Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	4604	cgmp or cyclic gmp	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:05
L2	206	1 near5 (detect$ or indicat$)	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:07
L3	1	2 near5 (fluorescen$ or fret)	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:08
L4	3	2 same (fluorescen$ or fret)	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:10
L5	177	1 same (fluorescen$ or fret)	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:10
L6	14	1 near5 (fluorescen$ or fret)	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:27
L7	9	2 and 5	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:27

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	4604	cgmp or cyclic gmp	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:05
L2	206	1 near5 (detect$ or indicat$)	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:07
L3	1	2 near5 (fluorescen$ or fret)	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:08
L4	3	2 same (fluorescen$ or fret)	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:08

PGPUB-DOCUMENT-NUMBER: 20030049728

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030049728 A1

TITLE: Nucleic acid sequences encoding capsaicin receptor and capsaicin receptor-related polypeptides and uses thereof

PUBLICATION-DATE: March 13, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Julius, David J.	San Francisco	CA	US	
Caterina, Michael J.	Mill Valley	CA	US	
Brake, Anthony J.	Berkeley	CA	US	

APPL-NO: 09/ 978303

DATE FILED: October 15, 2001

RELATED-US-APPL-DATA:

child 09978303 A1 20011015

parent continuation-of 09235451 19990122 US GRANTED

parent-patent 6335180 US

child 09978303 A1 20011015

parent continuation-of 08915461 19970820 US ABANDONED

non-provisional-of-provisional 60072151 19980122 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
US	PCT/US98/17466	1998US-PCT/US98/17466	August 20, 1998

US-CL-CURRENT: 435/69.1, 435/320.1 , 435/325 , 530/350 , 536/23.2

ABSTRACT:

The present invention features vanilloid receptor polypeptides and vanilloid receptor-related polypeptides, specifically the capsaicin receptor subtypes VR1 and VR2 (VRRP-1), as well as the encoding polynucleotide sequences. In related aspects the invention features expression vectors and host cells comprising such polynucleotides. In other related aspects, the invention features transgenic animals having altered capsaicin receptor expression, due to, for example, the presence of an exogenous wild-type or modified capsaicin receptor-encoding polynucleotide sequence. The present invention also relates to antibodies that bind specifically to a capsaicin receptor polypeptide, and methods for producing these polypeptides. Further, the invention provides methods for using capsaicin receptor, including methods for screening candidate agents for activity as agonists or antagonists of capsaicin receptor activity, as well as assays to determine the amount of a capsaicin receptor-activating agent in a sample. In other related aspects, the invention provides methods for the use of the capsaicin receptor for the diagnosis and treatment of human

e and painful syndromes.

S-REFERENCES TO RELATED APPLICATIONS

This application is a continuation-in-part of 1) U.S. provisional application serial No.60/072,151, filed Jan. 22, 1998; and 2) U.S. application Ser. No. 08/915,461, filed Aug. 20, 1997; and 3) PCT ational application PCT/US98/17466, filed Aug. 20, 1998, each of which ations are incorporated herein by reference.

-- KWIC ---------

Description Paragraph - DETX (94):
6] Preferably, capsaicin receptor-binding compounds are screened for stic or antagonist action in a functional assay that monitors a biological y associated with capsaicin receptor function such as effects upon ellular levels of cations in a capsaicin receptor-expressing host cell (e cium, magnesium, guanidinium, cobalt, potassium, cesium, sodium, and e, preferably calcium), ligand-activated conductances, cell death (i.e., tor-mediated cell death which can be monitored using, e.g., morphological s, chemical assays, or immunological assays), depolarization of the icin receptor-expressing cells (e.g., using fluorescent voltage-sensitive second messenger production (e.g., through detection of changes in GMP levels (see, e.g., Wood et al. 1989 J. Neurochem. 53:1203-1211), can be detected by radioimmunoassay or ELISA), calcium-induced reporter expression (see, e.g., Ginty 1997 Neuron 18:183-186), or other readily able biological activity associated with capsaicin receptor activity or ion of capsaicin receptor activity. Preferably, the functional assay is upon detection of a biological activity of capsaicin receptor that can be ed using high-throughput screening of multiple samples simultaneously, a functional assay based upon detection of a change in fluorescence which n is associated with a change in capsaicin receptor activity. Such onal assays can be used to screen candidate agents for activity as either icin receptor agonists or antagonists.

PGPUB-DOCUMENT-NUMBER: 20020137115

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020137115 A1

TITLE: Cgmp- visualizing probe and a method of detecting and quantifying of cgmp by using the same

PUBLICATION-DATE: September 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Umezawa, Yoshio	Tokyo		JP	
Sato, Moritoshi	Tokyo		JP	
Ozawa, Takeaki	Chiba		JP	

APPL-NO: 10/ 070131

DATE FILED: April 1, 2002

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	2000-202730	2000JP-2000-202730	July 4, 2000

PCT-DATA:
APPL-NO: PCT/JP01/05631
DATE-FILED: Jun 29, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/15, 435/194

ABSTRACT:

As a cGMP-visualizing probe capable of detecting and quantifying cGMP easily and accurately even in vivo and for a method of detecting and quantifying cGMP by using the same, a cGMP-visualizing probe comprising a polypeptide, which binds specifically to cGMP, and two chromophores having different fluorescence wavelengths each linked respectively to the two terminals of said polypeptide is provided.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):
As a cGMP-visualizing probe capable of detecting and quantifying cGMP easily and accurately even in vivo and for a method of detecting and quantifying cGMP by using the same, a cGMP-visualizing probe comprising a polypeptide, which binds specifically to cGMP, and two chromophores having different fluorescence wavelengths each linked respectively to the two terminals of said polypeptide is provided.

Summary of Invention Paragraph - BSTX (13):
[0010] Fifthly, the invention of the present application provides a method

for detecting and quantifying cGMP. which comprises making the cGMP-visualizing probe coexist with cGMP; and measuring the change in the fluorescence wavelength.

Detail Description Paragraph - DETX (9):

[0030] That is, when the cGMP-visualizing probe of the invention of the present application coexists with cGMP, the cGMP-binding protein binds to cGMP to allow FRET to be generated by the fluorescent chromophores at the N- and C-terminals thereof, thus causing a change in fluorescence wavelength. Then, cGMP may be detected by measuring such fluorescence change by a variety of conventional chemical and/or biochemical analysis techniques. Further, the concentration of cGMP in a sample solution may also be quantified by previously calibrating the relationship between fluorescence intensity and cGMP concentration.

Claims Text - CLTX (5):

5. A method for detecting and quantifying cGMP, which comprises: making the cGMP-visualizing probe of any one of claims 1 to 4 coexist with cGMP; and measuring the change in the fluorescence wavelength.

US-PAT-NO: 6335180

DOCUMENT-IDENTIFIER: US 6335180 B1

TITLE: Nucleic acid sequences encoding capsaicin receptor and uses thereof

DATE-ISSUED: January 1, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Julius; David J.	San Francisco	CA	N/A	N/A
Caterina; Michael J.	Mill Valley	CA	N/A	N/A
Brake; Anthony J.	Berkeley	CA	N/A	N/A

APPL-NO: 09/ 235451

DATE FILED: January 22, 1999

PARENT-CASE:

CROSS-REFERENCES TO RELATED APPLICATIONS

This application is a continuation-in-part of: 1) U.S. provisional patent application Ser. No. 60/072,151, filed Jan. 22, 1998; and 2) U.S. patent application Ser. No. 08/915,461, filed Aug. 20, 1997; now abandoned, and 3) PCT international application PCT/US98/17466, filed Aug. 20, 1998, each of which applications are incorporated herein by reference.

US-CL-CURRENT: 435/69.1, 435/252.3 , 435/320.1 , 536/23.5

ABSTRACT:

The present invention features vanilloid receptor polypeptides and vanilloid receptor-related polypeptides, specifically the capsaicin receptor subtypes VR1 and VR2 (VRRP-1), as well as the encoding polynucleotide sequences. In related aspects the invention features expression vectors and host cells comprising such polynucleotides. In other related aspects, the invention features transgenic animals having altered capsaicin receptor expression, due to, for example, the presence of an exogenous wild-type or modified capsaicin receptor-encoding polynucleotide sequence. The present invention also relates to antibodies that bind specifically to a capsaicin receptor polypeptide, and methods for producing these polypeptides. Further, the invention provides methods for using capsaicin receptor, including methods for screening candidate agents for activity as agonists or antagonists of capsaicin receptor activity, as well as assays to determine the amount of a capsaicin receptor-activating agent in a sample. In other related aspects, the invention provides methods for the use of the capsaicin receptor for the diagnosis and treatment of human disease and painful syndromes.

16 Claims, 28 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

---------- KWIC ---------

Detailed Description Text - DETX (96):

Preferably, capsaicin receptor-binding compounds are screened for agonistic or antagonist action in a functional assay that monitors a biological activity associated with capsaicin receptor function such as effects upon intracellular levels of cations in a capsaicin receptor-expressing host cell (e.g., calcium, magnesium, guanidinium, cobalt, potassium, cesium, sodium, and choline, preferably calcium), ligand-activated conductances, cell death (i.e., receptor-mediated cell death which can be monitored using, e.g., morphological assays, chemical assays, or immunological assays), depolarization of the capsaicin receptor-expressing cells (e.g., using fluorescent voltage-sensitive dyes), second messenger production (e.g., through detection of changes in cyclic GMP levels (see, e.g., Wood et al. 1989 J. Neurochem. 53:1203-1211), which can be detected by radioimmunoassay or ELISA), calcium-induced reporter gene expression (see, e.g., Ginty 1997 Neuron 18:183-186), or other readily assayable biological activity associated with capsaicin receptor activity or inhibition of capsaicin receptor activity. Preferably, the functional assay is based upon detection of a biological activity of capsaicin receptor that can be assayed using high-throughput screening of multiple samples simultaneously, e.g., a functional assay based upon detection of a change in fluorescence which in turn is associated with a change in capsaicin receptor activity. Such functional assays can be used to screen candidate agents for activity as either capsaicin receptor agonists or antagonists.

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	4604	cgmp or cyclic gmp	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:05
L2	206	1 near5 (detect$ or indicat$)	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:07
L3	1	2 near5 (fluorescen$ or fret)	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:08
L4	3	2 same (fluorescen$ or fret)	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:10
L5	177	1 same (fluorescen$ or fret)	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:10
L6	14	1 near5 (fluorescen$ or fret)	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:10

PGPUB-DOCUMENT-NUMBER: 20030228603

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030228603 A1

TITLE: Compositions selective for caffeine or aspartame and methods of using same

PUBLICATION-DATE: December 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Cload, Sharon T.	Cambridge	MA	US	
Ferguson, Alicia	Somerville	MA	US	

APPL-NO: 10/ 406903

DATE FILED: April 3, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60370266 20020405 US

non-provisional-of-provisional 60398858 20020725 US

US-CL-CURRENT: 435/6, 536/23.1

ABSTRACT:

Compositions which recognize and report on the concentration of caffeine or aspartame target molecules. The invention further relates to methods of using the compositions to monitor the presence or concentration of such targets in a variety of samples, including those samples to be ingested, such as beverages, e.g., coffee or soft drinks.

RELATED APPLICATIONS

[0001] This application claims priority to provisional patent application U.S. S. No. 60/370,266 filed on Apr. 5, 2002, which is incorporated herein by reference in its entirety.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (30):

[0043] FIG. 29 depicts the structure of the cGMP-dependent hammerhead construct used for the FRET assay.

Detail Description Paragraph - DETX (334):

[0384] Various sequence derivatives of the original hammerhead construct were synthesized and, following periodate oxidation, 3'-labelled with fluorescein thiosemicarbazide (FAM). Hybridization with dabcyl, tamra or QSY-7 modified quencher oligonucleotides constituted a set of FRET NASM constructs with different spatial arrangements of dye/quencher combinations (See FIGS. 6-10). Analysis of the fluorescence signals in response to the addition of cGMP revealed the most suitable structure to be that shown in FIG. 29.

Detail Description Paragraph - DETX (375):
[0421] Data indicating FRET donor signal of the immobilized, modified cGMP NASM reaction with cGMP is shown in FIG. 45.

PGPUB-DOCUMENT-NUMBER: 20030198700

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030198700 A1

TITLE: Personal care composition containing leghemoglobin

PUBLICATION-DATE: October 23, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Gruber, James V.	Somerville	NJ	US	

APPL-NO: 10/ 366231

DATE FILED: February 13, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60357544 20020215 US

US-CL-CURRENT: 424/773, 424/443 , 424/780 , 514/2 , 514/54

ABSTRACT:

A personal care composition comprising leghemoglobin and at least one preservative selected from the group consisting of alcohols, glycols, parabens, hydantoins, quaternary nitrogen-containing compounds, isothiazolinones, aldehyde-releasing agents, and halogenated compounds. Preferably, the leghemoglobin is a nitrogen fixation root nodule extract providing a leghemoglobin concentration in the composition of between 0.0001% and about 10% based upon the total weight of the composition. Also disclosed is a method for preparing the personal care composition.

---------- KWIC ---------

Detail Description Paragraph - DETX (59):

[0078] The catchpoint cGMP assay is a competitive fluorescence based ELISA assay. In this type of assay, as the amount of CGMP in a sample increases, the fluorescent signal will decrease. The assay procedure is as follows:

PGPUB-DOCUMENT-NUMBER: 20030170767

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030170767 A1

TITLE: Fluorescent protein sensors of post-translational modifications

PUBLICATION-DATE: September 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Cubitt, Andrew B.	San Diego	CA	US	

APPL-NO: 10/ 293580

DATE FILED: November 12, 2002

RELATED-US-APPL-DATA:

child 10293580 A1 20021112

parent continuation-of 09129192 19980724 US GRANTED

parent-patent 6495664 US

US-CL-CURRENT: 435/15, 435/23 , 435/320.1 , 435/325 , 435/69.1 , 530/350 , 536/23.5

ABSTRACT:

The present invention includes a fluorescent compound that can detect an activity. such as an enzymatic activity, and exhibits quenching. The fluorescent compound can include a fluorescent protein, such as an Aequorea-related green fluorescent protein. The fluorescent compound can include a substrate site for an enzymatic activity such as a kinase activity, a phosphatase activity, a protease activity, and a glycosylase activity The fluorescent compound of the present invention can be used to detect such enzymatic activities in samples, such as biological samples, including cells. The present invention also includes nucleic acids that encode the fluorescent compounds of the present inventions, and cells that include such nucleic acids or fluorescent compounds.

---------- KWIC --------

Brief Description of Drawings Paragraph - DRTX (62):

[0067] For example, a fluorescent protein substrate selective for phosphorylation by cGMP-dependent protein kinase can include the following consensus phosphorylation recognition motif sequence: BKISASEFDRPLR (SEQ ID NO:5), where B represents either lysine (K) or arginine (R), and the first S is the site of phosphorylation (Colbran et al, J. Biol. Chem. 267:9589-9594 (1992)). The residues DRPLR (SEQ ID NO:6) are less important than the phenylalanine (F) just preceding them for specific recognition by cGMP-dependent protein kinase in preference to cAMP-dependent protein kinase.

PGPUB-DOCUMENT-NUMBER: 20020137115

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020137115 A1

TITLE: Cgmp- visualizing probe and a method of detecting and quantifying of cgmp by using the same

PUBLICATION-DATE: September 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Umezawa, Yoshio	Tokyo		JP	
Sato, Moritoshi	Tokyo		JP	
Ozawa, Takeaki	Chiba		JP	

APPL-NO: 10/ 070131

DATE FILED: April 1, 2002

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	2000-202730	2000JP-2000-202730	July 4, 2000

PCT-DATA:
APPL-NO: PCT/JP01/05631
DATE-FILED: Jun 29, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/15, 435/194

ABSTRACT:

As a cGMP-visualizing probe capable of detecting and quantifying cGMP easily and accurately even in vivo and for a method of detecting and quantifying cGMP by using the same, a cGMP-visualizing probe comprising a polypeptide, which binds specifically to cGMP, and two chromophores having different fluorescence wavelengths each linked respectively to the two terminals of said polypeptide is provided.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):

As a cGMP-visualizing probe capable of detecting and quantifying cGMP easily and accurately even in vivo and for a method of detecting and quantifying cGMP by using the same, a cGMP-visualizing probe comprising a polypeptide, which binds specifically to cGMP, and two chromophores having different fluorescence wavelengths each linked respectively to the two terminals of said polypeptide is provided.

Summary of Invention Paragraph - BSTX (9):

[0006] To solve the problem described above, the invention of the present

application:first provides a cGMP-visualizing probe, comprising a polypeptide that binds specifically to cGMP and two chromophores with different fluorescence wavelengths, which are each linked to the two terminals of the polypeptide.

Summary of Invention Paragraph - BSTX (12):
[0009] Fourthly, the invention of the present application provides the cGMP-visualizing probe, wherein the chromophores are cyan fluorescent protein linked to the N-terminal of the polypeptide and yellow fluorescent protein linked to the C-terminal of the polypeptide.

Summary of Invention Paragraph - BSTX (13):
[0010] Fifthly, the invention of the present application provides a method for detecting and quantifying cGMP. which comprises making the cGMP-visualizing probe coexist with cGMP; and measuring the change in the fluorescence wavelength.

Detail Description Paragraph - DETX (8):
[0029] As the sites transducing the molecular recognition event to an optical change in the cGMP-visualizing probe of the invention of the present application, two fluorescent chromophores each having a different fluorescence wavelength are linked respectively to the two terminals of the polypeptide binding specifically to cGMP. AS such fluorescent chromophores, cyan fluorescent protein (CFP) i.e. a blue-shifted mutant of green fluorescent protein (GFP) and yellow fluorescent protein (YFP) i.e. a red-shifted mutant of GFP are preferably selected. By linking CFP to the N-terminal of the polypeptide binding specifically to cGMP and YFP to the C-terminal thereof, the two act, respectively as donor and acceptor to generate FRET.

Detail Description Paragraph - DETX (9):
[0030] That is, when the cGMP-visualizing probe of the invention of the present application coexists with cGMP, the cGMP-binding protein binds to cGMP to allow FRET to be generated by the fluorescent chromophores at the N- and C-terminals thereof, thus causing a change in fluorescence wavelength. Then, cGMP may be detected by measuring such fluorescence change by a variety of conventional chemical and/or biochemical analysis techniques. Further, the concentration of cGMP in a sample solution may also be quantified by previously calibrating the relationship between fluorescence intensity and cGMP concentration.

Detail Description Paragraph - DETX (36):
[0048] The CGY-expressing CHO-K1 cells prepared in Example 1 were stimulated with 8-Br-cGMP known as a cell membrane-permeable and phosphodiesterase-resistant-analogue of cGMP, and the fluorescence was measured under a fluorescence microscope.

PGPUB-DOCUMENT-NUMBER: 20020110890

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020110890 A1

TITLE: Use of the regulatory subunit of the cAMP dependent protein kinase (PKA) from dictyostelium for cAMP measurements

PUBLICATION-DATE: August 15, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Reymond, Christophe D.	Prilly		CH	

APPL-NO: 10/ 119941

DATE FILED: April 11, 2002

RELATED-US-APPL-DATA:

child 10119941 A1 20020411

parent division-of 09586605 20000602 US PENDING

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
EP	992017848	1999EP-992017848	June 4, 1999

US-CL-CURRENT: 435/194, 435/320.1 , 435/410 , 435/69.1 , 536/23.2

ABSTRACT:

The invention relates to the use of the regulatory subunit (R) of the CAMP dependent protein kinase (PKA) from Dictyostelium discoideum for cAMP detection. It includes constructs for expression of the R-subunit in E. coli and fusion to green fluorescent proteins (GFP). Fluorescence energy transfer is used as a way to monitor cAMP binding, either by using fluorescently labelled cAMP or cGMP, or by using mutant GFPs with modified absorption and emission spectra. FRET changes upon cAMP binding will allow measurement of cAMP level either in vitro or within living cells.

---------- KWIC --------

Abstract Paragraph - ABTX (1):

The invention relates to the use of the regulatory subunit (R) of the CAMP dependent protein kinase (PKA) from Dictyostelium discoideum for cAMP detection. It includes constructs for expression of the R-subunit in E. coli and fusion to green fluorescent proteins (GFP). Fluorescence energy transfer is used as a way to monitor cAMP binding, either by using fluorescently labelled cAMP or cGMP, or by using mutant GFPs with modified absorption and emission spectra. FRET changes upon cAMP binding will allow measurement of cAMP level either in vitro or within living cells.

Summary of Invention Paragraph - BSTX (19):

[0017] In the present invention, methods and compositions are provided for

producing Dictyostelium R-subunits in E. coli. DNA constructs allowing the expression of fusion proteins in E. coli are described in which donor and/or acceptor GFPs are inserted at particular locations within the R subunit allowing fluorescence energy transfer (FRET). Evidences for the occurrence of PRET are presented either between fluorescent cAMP or cGMP, or between acceptor and donor GFPs. FRET in modified upon cAMP binding Thus the GFP-R fusion proteins presented can be applied for the measurement of cAMP concentration.

Detail Description Paragraph - DETX (22):
[0045] The fluorescent nucleotide may be selected from cGMP, (8-[[2-[(Fluoresceinylthio-ureido)amino]ethyl]thio]guanosine-3',5'-cyclic monophosphate, CAMP and (8-[[2-[(Fluoresceinylthio-ureido)amino]ethyl]thio]adenosine-3',5"-cyclic monophosphate.

Detail Description Paragraph - DETX (35):
[0058] Fluorescently labelled cGMP (8-[[2-[(Fluoreneoinylthioureido)amino]-ethyl]thio] guanosine-3', 5'-cyclic, Biolog) was added to purified P26-OPP and fluorescence emission recorded while exciting at 433 nm. Fluorescence intensity decreased at 475 nm while it is increased at 520 nm, corresponding to the maximum of fluorescence for the fluorescent 8-fluo-cGMP (FIG. 2, open squares). A similar spectrum was obtained when using fluorescently labelled cAMP (8-[[2-[(Fluoreaceinylthioureido)amino]- ethyl]thio] adenozine-3',5'-cyclic monophosphate). Excitation at 433 nm of 8-fluo-cGMP alone gives almost undetectable emission at 520 nm, since excitation of 8-fluo-cGMP occurs around 494 nm. Those results indicate that FRET occurs between the w7-GFP fused to the truncated R-subunit and 9-fluo-cGMP.

Detail Description Paragraph - DETX (37):
[0060] The concentration of labelled 8-fluo-cGMP was varied while recording fluorescence changes (FIG. 3, inset). Fluorescence at 475 nm varied most and was thus used for the measurement of FRET variation A simple quadratic relation was obtained when plotting fluorescence intensity at 475 nm versus 8-fluo-cGMP concentration, allowing to measure an apparent dissociation constant, K.sub.D, of about 80.+-.10 nM (FIG. 3).

PGPUB-DOCUMENT-NUMBER: 20020061546

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020061546 A1

TITLE: Assays for protein kinases using fluorescent protein substrates

PUBLICATION-DATE: May 23, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Tsien, Roger Y.	La Jolla	CA	US	
Cubitt, Andrew B.	San Diego	CA	US	

APPL-NO: 09/ 884681

DATE FILED: June 19, 2001

RELATED-US-APPL-DATA:

child 09884681 A1 20010619

parent continuation-of 09263975 19990305 US GRANTED

parent-patent 6248550 US

child 09263975 19990305 US

parent continuation-of 08679865 19960716 US GRANTED

parent-patent 5912137 US

US-CL-CURRENT: 435/15, 435/194

ABSTRACT:

This invention provides assays for protein kinase activity using fluorescent proteins engineered to include sequences that can be phosphorylated by protein kinases. The proteins exhibit different fluorescent properties in the non-phosphorylated and phosphorylated states.

---------- KWIC ---------

Detail Description Paragraph - DETX (35):

[0063] For example, a fluorescent protein substrate selective for phosphorylation by cGMP-dependent protein kinase can include the following consensus sequence: BKISASEFDR PLR (SEQ ID NO:5), where B represents either lysine (K) or arginine (R), and the first S is the site of phosphorylation (Colbran et al. (1992) J. Biol. Chem. 267: 9589-9594). The residues DRPLR (SEQ ID NO:6) are less critical than the phenylalanine (F) just preceding them for specific recognition by cGMP-dependent protein kinase in preference to cAMP-dependent protein kinase.

US-PAT-NO: 6573059

DOCUMENT-IDENTIFIER: US 6573059 B1

TITLE: Use of the regulatory subunit of the camp dependent protein kinase (PKA) from dictyostelium for camp measurements

DATE-ISSUED: June 3, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Reymond; Christophe D.	Prilly	N/A	N/A	CH

APPL-NO: 09/ 586605

DATE FILED: June 2, 2000

US-CL-CURRENT: 435/15, 435/194 , 435/252.3 , 435/320.1 , 435/325 , 435/69.1 , 435/69.7 , 536/23.2 , 536/23.4

ABSTRACT:

The invention relates to the use of the regulatory subunit (R) of the cAMP dependent protein kinase (PKA) from Dictyostelium discoideum for cAMP detection. It includes constructs for expression of the R-subunit in E. coli and fusion to green fluorescent proteins (GFP). Fluorescence energy transfer is used as a way to monitor cAMP binding, either by using fluorescently labelled cAMP or cGMP, or by using mutant GFPs with modified absorption and emission spectra. FRET changes upon cAMP binding will allow measurement of cAMP level either in vitro or within living cells.

13 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

---------- KWIC ---------

Abstract Text - ABTX (1):

The invention relates to the use of the regulatory subunit (R) of the cAMP dependent protein kinase (PKA) from Dictyostelium discoideum for cAMP detection. It includes constructs for expression of the R-subunit in E. coli and fusion to green fluorescent proteins (GFP). Fluorescence energy transfer is used as a way to monitor cAMP binding, either by using fluorescently labelled cAMP or cGMP, or by using mutant GFPs with modified absorption and emission spectra. FRET changes upon cAMP binding will allow measurement of cAMP level either in vitro or within living cells.

Brief Summary Text - BSTX (19):

In the present invention, methods and compositions are provided for producing Dictyostelium R-subunits in E. coli. DNA constructs allowing the expression of fusion proteins in E. coli are described in which donor and/or acceptor GFPs are inserted at particular locations within the R subunit allowing fluorescence energy transfer (FRET). Evidences for the occurrence of

FRET are presented either between fluorescent cAMP or cGMP, or between acceptor and donor GFPs. FRET in modified upon cAMP binding Thus the GFP-R fusion proteins presented can be applied for the measurement of cAMP concentration.

Detailed Description Text - DETX (17):

The fluorescent nucleotide may be selected from cGMP, (8-[[2-[(Fluoresceinylthio-ureido)amino]ethyl]thio]guanosine-3', 5'-cyclic monophosphate, cAMP and (8-[[2-[(Fluoresceinylthio-ureido)amino]ethyl]thio]adenosine-3', 5'-cyclic monophosphate.

Detailed Description Text - DETX (27):

Fluorescently labelled cGMP (8-[[2-[(Fluoresceinylthioureido)amino]ethyl]thio] guanosine-3', 5'-cyclic, Biolog) was added to purified R26-GFP and fluorescence emission recorded while exciting at 433 nm. Fluorescence intensity decreased at 475 nm while it increased at 520 nm, corresponding to the maximum of fluorescence for the fluorescent 8-fluo-cGMP (FIG. 2, open squares). A similar spectrum was obtained when using fluorescently labelled cAMP (8-[[2-[(Fluoresceinylthioureido)amino]ethyl]thio]adenosine-3',5'-cyclic monophosphate). Excitation at 433 nm of 8-fluo-cGMP alone gives almost undetectable emission at 520 nm, since excitation of 8-fluo-cGMP occurs around 494 nm. These results indicate that FRET occurs between the w7-GFP fused to the truncated R-subunit and 8-fluo-cGMP.

Detailed Description Text - DETX (29):

The concentration of labelled 8-fluo-cGMP was varied while recording fluorescence changes (FIG. 3, inset). Fluorescence at 475 nm varied most and was thus used for the measurement of FRET variation. A simple quadratic relation was obtained when plotting fluorescence intensity at 475 nm versus 8-fluo-cGMP concentration, allowing to measure an apparent dissociation constant, K.sub.D, of about 80.+-.10 nM (FIG. 3).

US-PAT-NO: 6495664

DOCUMENT-IDENTIFIER: US 6495664 B1

TITLE: Fluorescent protein sensors of post-translational modifications

DATE-ISSUED: December 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cubitt; Andrew B.	San Diego	CA	N/A	N/A

APPL-NO: 09/ 129192

DATE FILED: July 24, 1998

US-CL-CURRENT: 530/350, 435/4 , 530/300

ABSTRACT:

The present invention includes a fluorescent compound that can detect an activity, such as an enzymatic activity, and exhibits quenching. The fluorescent compound can include a fluorescent protein, such as an Aequorea-related green fluorescent protein. The fluorescent compound can include a substrate site for an enzymatic activity such as a kinase activity, a phosphatase activity, a protease activity, and a glycosylase activity. The fluorescent compound of the present invention can be used to detect such enzymatic activities in samples, such as biological samples, including cells. The present invention also includes nucleic acids that encode the fluorescent compounds of the present inventions, and cells that include such nucleic acids or fluorescent compounds.

23 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

---------- KWIC --------

Detailed Description Text - DETX (41):

For example, a fluorescent protein substrate selective for phosphorylation by cGMP-dependent protein kinase can include the following consensus phosphorylation recognition motif sequence: BKISASEFDRPLR (SEQ ID NO:5), where B represents either lysine (K) or arginine (R), and the first S is the site of phosphorylation (Colbran et al, J. Biol. Chem. 267:9589-9594 (1992)). The residues DRPLR (SEQ ID NO:6) are less important than the phenylalanine (F) just preceding them for specific recognition by cGMP-dependent protein kinase in preference to cAMP-dependent protein kinase.

US-PAT-NO: 6248550

DOCUMENT-IDENTIFIER: US 6248550 B1

TITLE: Assays for protein kinases using fluorescent protein substrates

DATE-ISSUED: June 19, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsien; Roger Y.	La Jolla	CA	N/A	N/A
Cubitt; Andrew B.	San Diego	CA	N/A	N/A

APPL-NO: 09/ 263975

DATE FILED: March 5, 1999

PARENT-CASE:

This is a continuation of U.S. application Ser. No. 08/679,865, filed Jul. 16, 1996, now U.S. Pat. No. 5,912,137.

US-CL-CURRENT: 435/15, 435/194

ABSTRACT:

This invention provides assays for protein kinase activity using fluorescent proteins engineered to include sequences that can be phosphorylated by protein kinases. The proteins exhibit different fluorescent properties in the non-phosphorylated and phosphorylated states.

27 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

---------- KWIC ---------

Detailed Description Text - DETX (34):

For example, a fluorescent protein substrate selective for phosphorylation by cGMP-dependent protein kinase can include the following consensus sequence: BKISASEFDR PLR (SEQ ID NO:5), where B represents either lysine (K) or arginine (R), and the first S is the site of phosphorylation (Colbran et al. (1992) J. Biol. Chem. 267: 9589-9594). The residues DRPLR (SEQ ID NO:6) are less critical than the phenylalanine (F) just preceding them for specific recognition by cGMP-dependent protein kinase in preference to cAMP-dependent protein kinase.

US-PAT-NO: 5925558

DOCUMENT-IDENTIFIER: US 5925558 A

TITLE: Assays for protein kinases using fluorescent protein substrates

DATE-ISSUED: July 20, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsien; Roger Y.	La Jolla	CA	N/A	N/A
Cubitt; Andrew B.	San Diego	CA	N/A	N/A

APPL-NO: 08/ 680876

DATE FILED: July 16, 1996

US-CL-CURRENT: 435/252.3, 435/320.1 , 435/325 , 435/440 , 536/23.1 , 536/23.4

ABSTRACT:

This invention provides assays for protein kinase activity using fluorescent proteins engineered to include sequences that can be phosphorylated by protein kinases. The proteins exhibit different fluorescent properties in the non-phosphorylated and phosphorylated states.

23 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

---------- KWIC ---------

Detailed Description Text - DETX (34):

For example, a fluorescent protein substrate selective for phosphorylation by cGMP-dependent protein kinase can include the following consensus sequence: BKISASEFDR PLR (SEQ ID NO:5), where B represents either lysine (K) or arginine (R), and the first S is the site of phosphorylation (Colbran et al. (1992) J. Biol. Chem. 267: 9589-9594). The residues DRPLR (SEQ ID NO:6) are less critical than the phenylalanine (F) just preceding them for specific recognition by cGMP-dependent protein kinase in preference to cAMP-dependent protein kinase.

US-PAT-NO: 5912137

DOCUMENT-IDENTIFIER: US 5912137 A

TITLE: Assays for protein kinases using fluorescent

DATE-ISSUED: June 15, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsien; Roger Y.	La Jolla	CA	N/A	N/A
Cubitt; Andrew B.	San Diego	CA	N/A	N/A

APPL-NO: 08/ 679865

DATE FILED: July 16, 1996

US-CL-CURRENT: 435/15, 530/350 , 530/352 , 536/23.4

ABSTRACT:

This invention provides assays for protein kinase activity using fluorescent proteins engineered to include sequences that can be phosphorylated by protein kinases. The proteins exhibit different fluorescent properties in the non-phosphorylated and phosphorylated states.

24 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

---------- KWIC ---------

Detailed Description Text - DETX (33):

For example, a fluorescent protein substrate selective for phosphorylation by cGMP-dependent protein kinase can include the following consensus sequence: BKISASEFDR PLR (SEQ ID NO:5), where B represents either lysine (K) or arginine (R), and the first S is the site of phosphorylation (Colbran et al. (1992) J. Biol. Chem. 267: 9589-9594). The residues DRPLR (SEQ ID NO:6) are less critical than the phenylalanine (F) just preceding them for specific recognition by cGMP-dependent protein kinase in preference to cAMP-dependent protein kinase.

US-PAT-NO: 5039797

DOCUMENT-IDENTIFIER: US 5039797 A
See image for Certificate of Correction

TITLE: 2'-O-(4-benzoyl)benzoyl nucleoside cyclic monophosphate

DATE-ISSUED: August 13, 1991

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Clack; James W.	Branford	CT	N/A	N/A
Stein; Peter J.	Guilford	CT	N/A	N/A

APPL-NO: 07/ 421441

DATE FILED: October 13, 1989

US-CL-CURRENT: 536/47, 536/26.12 , 536/26.13

ABSTRACT:

The invention is a 2'-O-(4-benzoyl)benzoyl nucleoside 3', 5'-cyclic monophosphate of formula (I) ##STR1## wherein R' is guanine or adenine. Also disclosed are topical pharmaceutical compositions and methods of using them for reducing intraocular pressure.

8 Claims, 8 Drawing figures

Exemplary Claim Number: 1,6

Number of Drawing Sheets: 8

---------- KWIC ---------

Other Reference Publication - OREF (7):
The Journal of Biological Chemistry, vol. 257, No. 22, pp. 13354-13358, 1982 New Fluorescent Analogs of cAMP and cGMP Available as Substrates for Cyclic Nucleotide Phosphodiesterase; Toshiaki Hiratsuka.

US-PAT-NO: 4797480

DOCUMENT-IDENTIFIER: US 4797480 A

TITLE: New biologically active fluorescent cyclic nucleotides

DATE-ISSUED: January 10, 1989

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sorbi; Robert T.	Parma	N/A	N/A	IT
Caretta; Antonio	Parma	N/A	N/A	IT
Cavaggioni; Andrea	Parma	N/A	N/A	IT
Marchesi Gastaldi; Liliana	Parma	N/A	N/A	IT

APPL-NO: 06/ 866354

DATE FILED: May 23, 1986

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
IT	42506 A/85	June 6, 1985

US-CL-CURRENT: 536/26.13, 536/26.12

ABSTRACT:

Sybthesis of new biologically active fluorescent cyclic nucleotides of general formula: ##STR1## wherein X=OH and Y=NH.sub.2 or X=NH.sub.2 and Y=H and L is a fluorescent group of the class of 5 (or 6)-thioacetamido-fluorescein or of 5(or 8)-(2 thioacetamido-ethyl)-amino-naphthalene -1-sulphonic acid.

11 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Other Reference Publication - OREF (2):
Antonio Caretta et al., "Binding Stoichiometry of a Fluorescent cGMP Analogue to Membranes of Retinal Rod Outer Segments", 1985, Eur. J. Biochem., 153(1), pp. 49-53.

US-PAT-NO: 4554271

DOCUMENT-IDENTIFIER: US 4554271 A
See image for Certificate of Correction

TITLE: Use of high doses of derivatives of 6.alpha.-methylprednisolone for the acute treatment of stroke syndrome

DATE-ISSUED: November 19, 1985

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Braughler; J. Mark	Kalamazoo	MI	N/A	N/A
Hall; Edward D.	Portage	MI	N/A	N/A

APPL-NO: 06/ 582987

DATE FILED: February 24, 1984

US-CL-CURRENT: 514/179

ABSTRACT:

Use of water soluble 21-dibasic esters of 1-dehydro-6.alpha.-methylhydrocortisone for the acute treatment of stroke syndrome in humans.

5 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (18):

The present study was undertaken to examine the ability of a single large intravenous dose of methylprednisolone (15, 30, or 60 mg/kg) to attenuate lipid peroxidation and enhance (Na.sup.+ +K.sup.+) -ATPase activity during the 1st hour after a 400 gm-cm injury to the cat spinal cord. The contusion injury was associated with a rise in the concentration of fluorescent lipid peroxy products in the injured segment at 1 hour. In addition, the accumulation of cyclic guanosine 3',5'-monophosphate (cyclic GMP), which was used as a new index of injury-induced free radical reactions, in the injured spinal segment was twice control levels. The injury-induced increase in fluorescence and cyclic GMP content in the confused spinal segment at 1 hour was completely prevented by the administration of 15 or 30 mg/kg of methylprednisolone at 30 minutes after injury. A 60-mg/kg dose, however, did not prevent the elevation in cyclic GMP. A concomitant examination of the acute effects of glucocorticoid administration on (Na.sup.+ +K.sup.+)-ATPase activity in the injured cord revealed a striking increase of enzyme activity after the 30-mg/kg dose, but a depression in activity with the 60-mg/kg dose. These results demonstrate that a single massive dose of methylprednisolone can beneficially reduce free-radical reactions and lipid peroxidation as well as enhance the activity of neuronal (Na.sup.+ +K.sup.+)-ATPase during the early phase after spinal cord contusion. The requirement for doses to be in the range of 15 to 30 mg/kg in order to produce these neurochemical changes is consistent with

other studies that have demonstrated significantly greater recovery and tissue preservation in spinal cord-injured animals treated with comparable doses of methylprednisolone soon after injury. These findings suggest the need for a rigorous approach to glucocorticoid therapy in central nervous system trauma.

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	4604	cgmp or cyclic gmp	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:05
L2	206	1 near5 (detect$ or indicat$)	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:07
L3	1	2 near5 (fluorescen$ or fret)	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:08
L4	3	2 same (fluorescen$ or fret)	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:10
L5	177	1 same (fluorescen$ or fret)	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:10
L6	14	1 near5 (fluorescen$ or fret)	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:27
L7	9	2 and 5	US-PGPUB; USPAT	ADJ	OFF	2004/06/07 09:27

PGPUB-DOCUMENT-NUMBER: 20040087539

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040087539 A1

TITLE: Method of treating conditions related to platelet activity

PUBLICATION-DATE: May 6, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Du, Xiaoping	Westmont	IL	US	

APPL-NO: 10/ 467387

DATE FILED: December 12, 2003

PCT-DATA:
APPL-NO: PCT/US02/03372
DATE-FILED: Feb 5, 2002
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 514/45, 514/263.3

ABSTRACT:

Methods of treating thrombotic and hemostatic conditions related to platelet activity are described herein. The methods of treating thrombotic and hemostatic conditions use active agents that modulate production of guanosine 3', 5' cyclic monophosphate (cGMP) or the function of cGMP-dependent protein kinase (PKG), and its downstream effectors, the ERK and p38 pathways.

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 60/267,326, filed Feb. 8, 2001.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (10):
[0010] The present investigation indicates that cGMP induces biphasic platelet response. In the early phase, cGMP enhances activation of integrin .alpha..sub.IIb.beta..sub.3 and integrin-dependent platelet aggregation induced by vWF and thrombin. It also was found that binding of vWF to the platelet vWF receptor induces and enhances cGMP level and activate platelets via the PKG-cRaf-MEK (mitogen-activated protein kinase/-extracellular signal-regulated kinase kinase)-ERK (extracellular signal-regulated kinase) pathway. Furthermore, the p38 mitogen-activated protein kinase (MAPK) pathway also is important in mediating PKG-dependent activation of platelets. See Z. Li et al., J. Biol. Chem., 276(45), pp. 42226-42232 (Nov. 9, 2001).

Detail Description Paragraph - DETX (95):

[0117] In particular, FIG. 1 shows that expression of recombinant PKG promotes GPIb-LX-mediated integrin activation. In FIG. 1A, CHO cells expressing GPLb-JX and integrin .alpha..sub.IIb.beta..sub.3 (123 cells) were transfected with cDNA encoding PKGI.alpha. or vector. Expression of PKG Ia in 123 cells was detected by Western blotting with an anti-human PKG I antibody (Insert), and recombinant PKG activity was determined in the absence or presence of 20 .mu.M 8-bromo-cGMP as previously described. Results are expressed as mean +SD (n=3). For FIGS. 1B and 1C, reconstitution of GPIb-IX-mediated integrin activation in CHO cells was published (see Ref. 13). Integrin activation in the reconstituted system is dependent upon specific binding of vWF to GPLb-IX. PKG- or vector-transfected cells were incubated with Oregon Green-labeled fibrinogen (Fg) (20 .mu.g/ml) and 1 mg/mi ristocetin (No cGMP) for 30 mm with (+vWF) or without (No vWF) adding 12 .mu.g/ml vWF. These cells were also incubated with Oregon Green-labeled Fg, ristocetin and 8-bromo-cGMP (+cGMP) with or without adding vWF. Nonspecific binding was estimated by adding RGDS which inhibits fibrinogen binding to integrins (Fg+RGDS). Cells were analyzed by flow cytometry. Quantitative results from three experiments are expressed as fibrinogen binding indices (total bound fluorescence (Fg)/nonspecifically bound fluorescence (Fg+RGDS)), and shown in FIG. 1B (mean .+-.SD). Plots from a representative experiment are shown in FIG. 1C. Note that vWF-stimulated fibrinogen binding was significantly increased in PKG-transfected cells and was further increased by the PKG stimulator, 8-bromo-cGMP.

PGPUB-DOCUMENT-NUMBER: 20030211040

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030211040 A1

TITLE: Phosphodiesterase activity and regulation of phosphodiesterase 1B-mediated signaling in brain

PUBLICATION-DATE: November 13, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Greengard, Paul	New York	NY	US	
Repaske, David	Loveland	OH	US	
Snyder, Gretchen	New York	NY	US	

APPL-NO: 10/ 233449

DATE FILED: September 3, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60316320 20010831 US

US-CL-CURRENT: 424/9.2, 435/4 , 435/7.2

ABSTRACT:

The present invention provides methods and compositions for modulating the activity of phosphodiesterase 1B (PDE1B) in intracellular signaling pathways, including but not limited to, dopamine D1 intracellular signaling pathways. The invention also provides methods and compositions for modulating the activities of intracellular signaling molecules, including, but not limited to, DARPP-32 and GluR1 AMPA receptor, via modulation of PDE1B. The invention also provides pharmaceutical compositions and methods of screening for compounds that modulate PDE1B activity. The invention also provides methods of treating or ameliorating the symptoms of a disorder, including but not limited to a PDE1B-related disorder or a dopamine D1 receptor intracellular signaling pathway disorder, by administering a modulator of PDE1B, preferably, but not limited to, an inhibitor of PDE1B or an agent that decreases the production of PDE1B.

RELATED APPLICATIONS

[0001] This application claims benefit, under 35 U.S.C. .sctn. 119(e), of U.S. provisional application No. 60/316,320, filed on Aug. 31, 2001, which is incorporated herein by reference in its entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX (126):

[0255] CaM-PDE activity may be assayed according to methods commonly known in the art. Phosphodiesterase cleavage of radioactively labeled cAMP or cGMP can be used to detect phosphodiesterase inhibitors. In one embodiment, the assay for phosphodiesterase activity relies on recovery of labeled adenosine following a two-step biochemical assay. In the first step, phosphodiesterase

hydrolyzes [.sup.3H]cyclic AMP to [.sup.3H]5'-AMP. In the second step, snake venom 5'-nucleotidase is added to convert the [.sup.3H]5'-AMP to [.sup.3H]adenosine. Labeled adenosine is separated from [.sup.31H]cyclic AMP by anion exchange or affinity chromatography and then detected by liquid scintillation counting. One such assay has been described for cAMP-specific phosphodiesterase PDE4 by Hansen and Beavo (1982, "Purification of two calcium/calmodulin-dependent forms of cyclic nucleotide phosphodiesterase by using conformation-specific monoclonal antibody chromatography." Proc Natl Acad Sci U S A 79(9): 2788-92), and modified for high throughput by Daniels and Alvarez (1996, "A semiautomated method for the assay of cyclic adenosine 5'-monophosphate phosphodiesterase." Anal Biochem 236(2): 367-9).

Detail Description Paragraph - DETX (139):

[0268] In one embodiment, the methods disclosed in Bader et al. (2001, A cGMP-dependent protein kinase assay for high throughput screening based on time-resolved fluorescence resonance energy transfer, Journal of Biomolecular Screening 6(4): 255-64) are used to determine activity of e.g., a phosphodiesterase, kinase or protein phosphatase. Bader et al. discloses a cGMP-dependent protein kinase assay for high throughput screening based on time-resolved fluorescence resonance energy transfer ("FRET"), which as, would be appreciated by one of skill in the art, may be adapted for assays of a phosphodiesterase or protein phosphatase. Samples containing the kinase of interest are exposed to ATP and a synthetic peptide substrate with a kinase-specific phosphorylation site and an amino-terminal biotin moiety. Phosphorylated peptide is detected using allophycocyanin-labeled streptavidin, a phosphopeptide specific antibody, and a Europium-chelate-labeled secondary antibody. Simultaneous binding of the streptavidin and the phosphospecific antibody to a phosphorylated substrate molecule brings the Europium chelate "donor" on the secondary antibody close enough to the allophycocyanin fluorophore "acceptor" for fluorescence resonance energy transfer to occur, measurable as a decrease in Europium emission at 615 nm and an increase in allophycocyanin emission at 665 nm wavelength. The Europium--allophycocyanin donor--acceptor pair is commonly used in order to take advantage of the long fluorescence lifetime of excited Europium, thus the signal is "time-resolved".

PGPUB-DOCUMENT-NUMBER: 20030166213

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030166213 A1

TITLE: Methods for identifying compounds that modulate disorders related to nitric oxide/ cGMP-dependent protein kinase signaling

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Greenspan, Ralph J.	Coronado	CA	US	
Shaw, Paul J.	San Diego	CA	US	

APPL-NO: 09/ 738630

DATE FILED: December 15, 2000

US-CL-CURRENT: 435/194, 424/9.2 , 435/6 , 536/23.2

ABSTRACT:

The invention provides a method of identifying a compound that modulates a disease associated with a nitric oxide/cGMP-dependent protein kinase network in a mammal. The method consists of (a) administering a test compound to an invertebrate; and (b) measuring a foraging behavior of the invertebrate, wherein a compound that modulates the foraging behavior of the invertebrate is characterized as a compound that modulates a disease associated with a nitric oxide/cGMP-dependent protein kinase network in a mammal.

---------- KWIC ---------

Detail Description Paragraph - DETX (56):

[0065] A compound used to contact the invertebrate can be identified as any molecule that potentially alters foraging. Additionally, a compound to be used in the methods of the invention can be identified based on presumed or predicted activity in ADHD, hypertension, or other disease associated with a NO/cGMP-dependent kinase network in a mammal as indicated for example by molecular properties, interactions observed at the molecular or cellular level, clinical evidence, or other empirical evidence known to one skilled in the art to be predictive of such activities.

Detail Description Paragraph - DETX (73):

[0082] Additionally, constructs containing the promoter of a gene associated with foraging behavior in an invertebrate or with ADHD, hypertension, or other disease associated with a nitric oxide/cGMP-dependent protein kinase network in a mammal can be functionally fused to a reporter gene (e.g. .beta.-galactosidase, green fluorescent protein, luciferase) using known methods, and used to generate transgenic invertebrates. Such transgenic invertebrates can be used in the methods of the invention wherein expression of the reporter gene can be a marker for expression of a polynucleotide that modulates ADHD, hypertension or other disease associated with a nitric oxide/cGMP-dependent protein kinase network in a mammal.

PGPUB-DOCUMENT-NUMBER: 20030049728

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030049728 A1

TITLE: Nucleic acid sequences encoding capsaicin receptor and capsaicin receptor-related polypeptides and uses thereof

PUBLICATION-DATE: March 13, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Julius, David J.	San Francisco	CA	US	
Caterina, Michael J.	Mill Valley	CA	US	
Brake, Anthony J.	Berkeley	CA	US	

APPL-NO: 09/ 978303

DATE FILED: October 15, 2001

RELATED-US-APPL-DATA:

child 09978303 A1 20011015

parent continuation-of 09235451 19990122 US GRANTED

parent-patent 6335180 US

child 09978303 A1 20011015

parent continuation-of 08915461 19970820 US ABANDONED

non-provisional-of-provisional 60072151 19980122 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
US	PCT/US98/17466	1998US-PCT/US98/17466	August 20, 1998

US-CL-CURRENT: 435/69.1, 435/320.1 , 435/325 , 530/350 , 536/23.2

ABSTRACT:

The present invention features vanilloid receptor polypeptides and vanilloid receptor-related polypeptides, specifically the capsaicin receptor subtypes VR1 and VR2 (VRRP-1), as well as the encoding polynucleotide sequences. In related aspects the invention features expression vectors and host cells comprising such polynucleotides. In other related aspects, the invention features transgenic animals having altered capsaicin receptor expression, due to, for example, the presence of an exogenous wild-type or modified capsaicin receptor-encoding polynucleotide sequence. The present invention also relates to antibodies that bind specifically to a capsaicin receptor polypeptide, and methods for producing these polypeptides. Further, the invention provides methods for using capsaicin receptor, including methods for screening candidate agents for activity as agonists or antagonists of capsaicin receptor activity, as well as assays to determine the amount of a capsaicin receptor-activating agent in a sample. In other related aspects, the invention provides methods for the use of the capsaicin receptor for the diagnosis and treatment of human

disease and painful syndromes.

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of 1) U.S. provisional patent application serial No.60/072,151, filed Jan. 22, 1998; and 2) U.S. patent application Ser. No. 08/915,461, filed Aug. 20, 1997; and 3) PCT international application PCT/US98/17466, filed Aug. 20, 1998, each of which applications are incorporated herein by reference.

---------- KWIC ---------

Detail Description Paragraph - DETX (94):

[0116] Preferably, capsaicin receptor-binding compounds are screened for agonistic or antagonist action in a functional assay that monitors a biological activity associated with capsaicin receptor function such as effects upon intracellular levels of cations in a capsaicin receptor-expressing host cell (e g., calcium, magnesium, guanidinium, cobalt, potassium, cesium, sodium, and choline, preferably calcium), ligand-activated conductances, cell death (i.e., receptor-mediated cell death which can be monitored using, e.g., morphological assays, chemical assays, or immunological assays), depolarization of the capsaicin receptor-expressing cells (e.g., using fluorescent voltage-sensitive dyes), second messenger production (e.g., through detection of changes in cyclic GMP levels (see, e.g., Wood et al. 1989 J. Neurochem. 53:1203-1211), which can be detected by radioimmunoassay or ELISA), calcium-induced reporter gene expression (see, e.g., Ginty 1997 Neuron 18:183-186), or other readily assayable biological activity associated with capsaicin receptor activity or inhibition of capsaicin receptor activity. Preferably, the functional assay is based upon detection of a biological activity of capsaicin receptor that can be assayed using high-throughput screening of multiple samples simultaneously, e.g., a functional assay based upon detection of a change in fluorescence which in turn is associated with a change in capsaicin receptor activity. Such functional assays can be used to screen candidate agents for activity as either capsaicin receptor agonists or antagonists.

PGPUB-DOCUMENT-NUMBER: 20020137115

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020137115 A1

TITLE: Cgmp- visualizing probe and a method of detecting and quantifying of cgmp by using the same

PUBLICATION-DATE: September 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Umezawa, Yoshio	Tokyo		JP	
Sato, Moritoshi	Tokyo		JP	
Ozawa, Takeaki	Chiba		JP	

APPL-NO: 10/ 070131

DATE FILED: April 1, 2002

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	2000-202730	2000JP-2000-202730	July 4, 2000

PCT-DATA:
APPL-NO: PCT/JP01/05631
DATE-FILED: Jun 29, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/15, 435/194

ABSTRACT:

As a cGMP-visualizing probe capable of detecting and quantifying cGMP easily and accurately even in vivo and for a method of detecting and quantifying cGMP by using the same, a cGMP-visualizing probe comprising a polypeptide, which binds specifically to cGMP, and two chromophores having different fluorescence wavelengths each linked respectively to the two terminals of said polypeptide is provided.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):

As a cGMP-visualizing probe capable of detecting and quantifying cGMP easily and accurately even in vivo and for a method of detecting and quantifying cGMP by using the same, a cGMP-visualizing probe comprising a polypeptide, which binds specifically to cGMP, and two chromophores having different fluorescence wavelengths each linked respectively to the two terminals of said polypeptide is provided.

Title - TTL (1):

Cgmp- visualizing probe and a method of detecting and quantifying of cgmp

by using the same

Summary of Invention Paragraph - BSTX (2):
[0001] The invention of the present application relates to a visualizing probe for detecting and quantifying cGMP and a method of detecting and quantifying cGMP by using the same. More specifically, the present invention relates to a cGMP-visualizing probe which can specifically bind to CGMP, thereby generating an optical change to enable detection and quantification of cGMP, and a method of detecting and quantifying cGMP by using the same.

Summary of Invention Paragraph - BSTX (5):
[0003] Conventional methods of detecting and quantifying cGMP include radioimmunoassays using a radioisotope-labeled compound. However, this method involves disrupting cells and detecting the binding of cellular lysates to the labeled compound to analyze total cGMP levels, and did not realize the accuracy required in cell-biology and pharmaceutics.

Summary of Invention Paragraph - BSTX (7):
[0005] The invention of the present application has been made in view of the circumstances as described above, and the object of the present invention is to Solve the problem in the prior art and to provide a cGMP-visualizing probe, which enables the easy and highly accurate detection and quantification of cGMP, even in vivo, as well as a method of detecting and quantifying cGMP by using the same.

Summary of Invention Paragraph - BSTX (9):
[0006] To solve the problem described above, the invention of the present application:first provides a cGMP-visualizing probe, comprising a polypeptide that binds specifically to cGMP and two chromophores with different fluorescence wavelengths, which are each linked to the two terminals of the polypeptide.

Summary of Invention Paragraph - BSTX (12):
[0009] Fourthly, the invention of the present application provides the cGMP-visualizing probe, wherein the chromophores are cyan fluorescent protein linked to the N-terminal of the polypeptide and yellow fluorescent protein linked to the C-terminal of the polypeptide.

Summary of Invention Paragraph - BSTX (13):
[0010] Fifthly, the invention of the present application provides a method for detecting and quantifying cGMP. which comprises making the cGMP-visualizing probe coexist with cGMP; and measuring the change in the fluorescence wavelength.

Summary of Invention Paragraph - BSTX (14):
[0011] Sixthly, the invention of the present application provides the method for detecting and quantifying cGMP, which comprises introducing a polynucleotide expressing the cGMP-visualizing probe into cells, thereby making the cGMP-visualizing probe coexist with cGMP.

Summary of Invention Paragraph - BSTX (15):
[0012] Seventhly, the invention of the present application provides the method for detecting and quantifying cGMP, which comprises introducing a polynucleotide expressing a cGMP-visualizing probe into cells and performing ontogenesis from the non-human animal totipotent cells, thereby making the cGMP-visualizing probe coexist with cGMP in every cell of the resultant animal or its offspring.

Detail Description Paragraph - DETX (3):

[0024] The detection and quantifcation of cGMP is enabled when the cGMP-visualizing probe of this invention coexists with cGMP; the site that binds specifically to cGMP (cGMP-binding site) binds to cGMP, causing the change of configuration of the coloring sites, which may be detected as an optical change.

Detail Description Paragraph - DETX (5):
[0026] Mammalian PKG I.alpha. is composed of two identical monomers each having four types of functional domains (dimerization domain, autoinhibitory domain, cGMP-binding domain and catalytic domain) as shown in FIG. 1. The dimerization domain located at the N-terminus is composed of a leucine/isoleucine zipper motif. In the absence of cGMP, PKG I.alpha. displays a kinase inactive closed conformation, in which its catalytic center is occupied by an autoinhibitory domain. Upon binding of PKG I.alpha. to cGMP, PKG I.alpha. displays an open conformation in which the autoinhibitory domain is removed from the catalytic center. Accordingly, if chromophores are linked to both terminals of PKG I.alpha., an optical change will occur upon binding of PKG I.alpha. to cGMP, thus allowing the optical detection of the binding to cGMP.

Detail Description Paragraph - DETX (7):
[0028] In the cGMP-visualizing probe of the invention of the present application, the site transducing the molecular recognition to an optical change may be chosen from various chromophores. In this transduction, the chromophores should generate a change in wavelength highly accurately by responding to the change in the stereostructure resulting from the binding of the cGMP-binding site to cGMP. In the field of biochemistry, there are a wide variety of generally used fluorescent chromophores including, as chromophores responding rapidly to a change in the stereostructure, those causing change in color by the generation of fluorescence resonance energy transfer (FRET).

Detail Description Paragraph - DETX (8):
[0029] As the sites transducing the molecular recognition event to an optical change in the cGMP-visualizing probe of the invention of the present application, two fluorescent chromophores each having a different fluorescence wavelength are linked respectively to the two terminals of the polypeptide binding specifically to cGMP. AS such fluorescent chromophores, cyan fluorescent protein (CFP) i.e. a blue-shifted mutant of green fluorescent protein (GFP) and yellow fluorescent protein (YFP) i.e. a red-shifted mutant of GFP are preferably selected. By linking CFP to the N-terminal of the polypeptide binding specifically to cGMP and YFP to the C-terminal thereof, the two act, respectively as donor and acceptor to generate FRET.

Detail Description Paragraph - DETX (9):
[0030] That is, when the cGMP-visualizing probe of the invention of the present application coexists with cGMP, the cGMP-binding protein binds to cGMP to allow FRET to be generated by the fluorescent chromophores at the N- and C-terminals thereof, thus causing a change in fluorescence wavelength. Then, cGMP may be detected by measuring such fluorescence change by a variety of conventional chemical and/or biochemical analysis techniques. Further, the concentration of cGMP in a sample solution may also be quantified by previously calibrating the relationship between fluorescence intensity and cGMP concentration.

Detail Description Paragraph - DETX (10):
[0031] In the invention of the present application, various methods are applicable for the cGMP-visualizing probe described above to coexist with cGMP. For example, a method wherein cells are disrupted, cGMP is eluted from the cells, and the cGMP-visualizing probe is added to the solution, to allow the

cGMP-visualizing probe to be coexistent with cGMP maybe applied. When the cGMP-visualizing probe is allowed to be coexistent with cGMP by such a method, CGMP can be detected and quantified in vitro.

Detail Description Paragraph - DETX (11):
[0032] In the present invention, by introducing an expression vector having the cGMP-visualizing probe integrated therein into individual cultured cells, the cGMP-visualizing probe may also be made to coexist with cGMP . For such a method, the expression vector, a plasmid vector for expression in animal cells is preferable. The introduction of such plasmid vectors into cells, may be accomplished by known methods such as electroporation, calcium phosphate method, liposome method and DEAE-dextran method. By using the method of introducing an expression vector having the cGMP-visualizing probe integrated therein into cells, cGMP and the cGMP-visualizing probe may coexist in the cells, thus enabling an in vivo method of detecting and quantifying cGMP without disrupting the cells.

Detail Description Paragraph - DETX (12):
[0033] Further, in the method of detecting and measuring cGMP by the invention of the present application, a polynucleotide expressing the cGMp-visualizing probe may be introduced into cells, and by ontogenesis, the non-human animal totipotent cells may be generated into an individual non-human animal, for the cGMP-visualizing probe to coexist with cGMP in every cell of the resultant animal or its offspring.

Detail Description Paragraph - DETX (20):
[0038] As shown in FIG. 1, cyan fluorescent protein (ECFP: F64L/S65T/Y66W/N1461/M153T/V163A/N212K) and yellow fluorescent protein (EYFP S65G/V68L/Q69K/S72A/T203Y), which are mutants of green fluorescent protein (EGFP: for example, Current Biology 6(2), 178-182, 1996) derived from fluorescent Aequorea Victoria, were linked respectively to the N- and C-terminals of cGMP-dependent protein kinase (PKG I.alpha.) by genetic engineering, to prepare a CFP-PKG I.alpha.-YFP fusion protein (referred to hereinafter as CGY).

Detail Description Paragraph - DETX (36):
[0048] The CGY-expressing CHO-K1 cells prepared in Example 1 were stimulated with 8-Br-cGMP known as a cell membrane-permeable and phosphodiesterase-resistant-analogue of cGMP, and the fluorescence was measured under a fluorescence microscope.

Detail Description Paragraph - DETX (40):
[0051] From these results, it was revealed that there is no significant emission ratio change of CGY-FL. It was also revealed that the fluorescence intensities of CFP and YFP were not affected by the addition of 8-Br-cGMP.

Detail Description Paragraph - DETX (44):
[0054] These results indicate that the fluorescence resonance energy transfer (FRET) between CFP and YFP increases upon binding of 8-BR-cGMP to CGY-Del1.

Detail Description Paragraph - DETX (60):
[0063] The cGMP-visualizing probes were used to detect intracellular cGMP generated upon stimulation of the living cells with nitrogen monoxide (NO).

Detail Description Paragraph - DETX (76):
[0077] From the foregoing results, it was confirmed that the response of CGY-Del1 in detecting cGMP is reversible, and by using the cGMP-visualizing probe of the invention of the present application, the fluctuating

concentration of cGMP in living cells can be measured.

Detail Description Paragraph - DETX (78):
[0078] As described in detail above, the present invention provides a cGMP-visualizingprobe that enables the easy detection and quantification of cGMP with high accurately even in vivo, as well as a method of detecting and quantifying cGMP by using the same.

Claims Text - CLTX (1):
1. A cGMP-visualizing probe, comprising: a polypeptide that binds specifically to cGMP; and two chromophores with different fluorescence wavelengths, which are each linked separately to the two terminals of the polypeptide.

Claims Text - CLTX (4):
4. The cGMP-visualizing probe of anyone of claims 1 to 3, wherein the chromophores are cyan fluorescent protein linked to the N-terminal of the polypeptide and yellow fluorescent protein linked to the C-terminal of the polypeptide.

Claims Text - CLTX (5):
5. A method for detecting and quantifying cGMP, which comprises: making the cGMP-visualizing probe of any one of claims 1 to 4 coexist with cGMP; and measuring the change in the fluorescence wavelength.

Claims Text - CLTX (6):
6. The method for detecting and quantifying cGMP of claim 5, which comprises introducing a polynucleotide expressing the cGMP-visualizing probe of any one of claims 1 to 4 into cells, whereby making the cGMP-visualizing probe coexist with cGMP.

Claims Text - CLTX (7):
7. The method for detecting and quantifying cGMP of claim 5, which comprises: introducing a polynucleotide expressing a cGMP-visualizing probe of any one of claims 1 to 4 into cells; and performing ontogenisis from the non-human animal totipotent cells. thereby making the cGMP-visualizing probe coexist with cGMP in every cell of the resultant animal or its offspring.

US-PAT-NO: 6335180

DOCUMENT-IDENTIFIER: US 6335180 B1

TITLE: Nucleic acid sequences encoding capsaicin receptor and uses thereof

DATE-ISSUED: January 1, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Julius; David J.	San Francisco	CA	N/A	N/A
Caterina; Michael J.	Mill Valley	CA	N/A	N/A
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APPL-NO: 09/ 235451

DATE FILED: January 22, 1999

PARENT-CASE:

CROSS-REFERENCES TO RELATED APPLICATIONS

This application is a continuation-in-part of: 1) U.S. provisional patent application Ser. No. 60/072,151, filed Jan. 22, 1998; and 2) U.S. patent application Ser. No. 08/915,461, filed Aug. 20, 1997; now abandoned, and 3) PCT international application PCT/US98/17466, filed Aug. 20, 1998, each of which applications are incorporated herein by reference.

US-CL-CURRENT: 435/69.1, 435/252.3 , 435/320.1 , 536/23.5

ABSTRACT:

The present invention features vanilloid receptor polypeptides and vanilloid receptor-related polypeptides, specifically the capsaicin receptor subtypes VR1 and VR2 (VRRP-1), as well as the encoding polynucleotide sequences. In related aspects the invention features expression vectors and host cells comprising such polynucleotides. In other related aspects, the invention features transgenic animals having altered capsaicin receptor expression, due to, for example, the presence of an exogenous wild-type or modified capsaicin receptor-encoding polynucleotide sequence. The present invention also relates to antibodies that bind specifically to a capsaicin receptor polypeptide, and methods for producing these polypeptides. Further, the invention provides methods for using capsaicin receptor, including methods for screening candidate agents for activity as agonists or antagonists of capsaicin receptor activity, as well as assays to determine the amount of a capsaicin receptor-activating agent in a sample. In other related aspects, the invention provides methods for the use of the capsaicin receptor for the diagnosis and treatment of human disease and painful syndromes.

16 Claims, 28 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

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Detailed Description Text - DETX (96):

Preferably, capsaicin receptor-binding compounds are screened for agonistic or antagonist action in a functional assay that monitors a biological activity associated with capsaicin receptor function such as effects upon intracellular levels of cations in a capsaicin receptor-expressing host cell (e.g., calcium, magnesium, guanidinium, cobalt, potassium, cesium, sodium, and choline, preferably calcium), ligand-activated conductances, cell death (i.e., receptor-mediated cell death which can be monitored using, e.g., morphological assays, chemical assays, or immunological assays), depolarization of the capsaicin receptor-expressing cells (e.g., using fluorescent voltage-sensitive dyes), second messenger production (e.g., through detection of changes in cyclic GMP levels (see, e.g., Wood et al. 1989 J. Neurochem. 53:1203-1211), which can be detected by radioimmunoassay or ELISA), calcium-induced reporter gene expression (see, e.g., Ginty 1997 Neuron 18:183-186), or other readily assayable biological activity associated with capsaicin receptor activity or inhibition of capsaicin receptor activity. Preferably, the functional assay is based upon detection of a biological activity of capsaicin receptor that can be assayed using high-throughput screening of multiple samples simultaneously, e.g., a functional assay based upon detection of a change in fluorescence which in turn is associated with a change in capsaicin receptor activity. Such functional assays can be used to screen candidate agents for activity as either capsaicin receptor agonists or antagonists.

US-PAT-NO: 6214888

DOCUMENT-IDENTIFIER: US 6214888 B1

TITLE: Dermatological compounds

DATE-ISSUED: April 10, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ren; Wu Yun	Germantown	MD	N/A	N/A
Brown; David A.	Ellicott City	MD	N/A	N/A

APPL-NO: 09/ 086547

DATE FILED: May 28, 1998

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of PCT/US98/05346 filed Mar. 18, 1998, which is a continuation-in-part of PCT/US97/16642 filed Sep. 18, 1997, which is a continuation-in-part of application Ser. No. 60/026,577 filed Sep. 18, 1996, of application Ser. No. 60/035,947 filed Jan. 21, 1997, of application Ser. No. 60/036,863 filed Feb. 4, 1997, and of application Ser. No. 60/048,597 filed Jun. 4, 1997.

US-CL-CURRENT: 514/729, 568/819 , 568/820

ABSTRACT:

The present invention relates to novel monocyclic and bicyclic monoterpene diols that stimulate melanogenesis in mammalian skin, hair, wool or fur, and, are useful for treating or preventing various skin and proliferative disorders, neurodegenerative diseases, and diseases regulated by the nitric oxide/cyclic GMP/protein kinase G pathway.

19 Claims, 1 Drawing figures

Exemplary Claim Number: 1,11

Number of Drawing Sheets: 1

---------- KWIC ---------

Detailed Description Text - DETX (13):

It has also been discovered that the present class of compounds stimulate cellular nitric oxide synthesis and have their action blocked by scavengers of nitric oxide (NO), and by inhibitors of cyclic guanosine monophosphate (cGMP) or inhibitors of cGMP-activated protein kinase (PKG). This indicates that these compounds act via the NO/cGMP/PKG signal transduction pathway. Unlike previous compounds like nitroglycerin and isosorbide dinitrate that stimulate this pathway by releasing NO upon reaction with intracellular sulfhydryl groups (Smith and Reynard, 1992, Pharmacology, W.B. Saunders Co., Philadelphia, Pa., pp. 626-31), the compounds of this invention appear to act by direct

stimulation of nitric oxide synthase (NOS) activity, thus generating NO de novo. Whereas depletion of intracellular sulfhydryl groups rapidly leads to tolerance and ineffectiveness of nitroglycerin and related compounds (Smith and Reynard, 1992), tolerance will not be acquired to the compounds of the present invention since they do not require the presence of sulfhydryl groups for generation. Thus, it is contemplated that the compounds of the present invention will provide a preferred alternative method of treatment for conditions presently treated by NO donors.

Detailed Description Text - DETX (38):

Discovery of the pathway through which the present compounds act also leads to methods for screening compounds for melanogenic activity and potency, or for their ability to reduce or suppress melanogenesis, based on measurement of generation of nitric oxide (NO) or measurement of nitric oxide synthesis (NOS) activity. Methods for measurement of NO or NOS include but are not limited to the following well known methods. Measurement of NO is usually based on the fact that NO rapidly decomposes to nitrate and nitrite in aqueous solution. Nitrate reductase is added to culture media or cell extracts to ensure complete conversion of nitrate to nitrite. Griess reagents (sulfanilamide and N-[1-naphthyl]-ethylenediamine) are then added to convert nitrite into a deep purple azo compound that absorbs maximally at 540 nm (Schmidt, et al., 1995, Biochemica 2:22). Reactions are typically carried out in a 96-well format with absorbances read on a microtiter plate reader. Alternatively, following conversion of nitrate to nitrite as described above, DAN reagent (2,3-diaminonaphthalene) is added followed by NaOH which converts nitrite into the fluorescent compound 1(H)-naphthotriazole. This is measured fluorimetrically with excitation at 365 nm and emission at 450 nm, typically in a 96-well format (Miles, et al., 1995, Methods 7:40). NOS activity is measured by adding [.sup.3 H]-arginine to intact tissues or protein extracts, and measuring release of .sup.3 H resulting from the conversion of arginine to citrulline during the enzymatic formation of NO by NOS (Baudouin and Tachon, 1996, J. Invest. Dermatol. 106:428-431). Alternatively, the production of cGMP or activity of PKG can be used as a screening tool. cGMP may be measured by commercially available immunoassay (see Romero-Graillet, et al., 1996, J. Biol. Chem. 271:28052-28056). PKG may be measured by cyclic GMP dependent kination of a primary histone target (see Hidaka, et al., Biochemistry 1984, 23, 5036-5041)

US-PAT-NO: 5521295

DOCUMENT-IDENTIFIER: US 5521295 A

TITLE: Nucleic acids encoding hybrid receptor molecules

DATE-ISSUED: May 28, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Pacifici; Robert E.	Thousand Oaks	CA	N/A	N/A
Thomason; Arlen R.	Thousand Oaks	CA	N/A	N/A

APPL-NO: 08/ 336708

DATE FILED: November 8, 1994

PARENT-CASE:

This application is a continuation of application Ser. No. 08/073,196, filed Jun. 7, 1993 abandoned which is hereby incorporated by reference.

US-CL-CURRENT: 536/23.4, 435/320.1 , 435/325 , 435/354 , 435/365 , 435/372 , 435/7.1 , 530/350

ABSTRACT:

Provided are hybrid receptor molecules wherein one domain of the receptor is derived from the cytokine superfamily of receptors and other domain is derived from a heterologous family of receptors. Also provided are methods for identifying ligands that bind to the hybrid receptor molecules.

5 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

---------- KWIC ---------

Detailed Description Text - DETX (9):
The term "biologically active" refers to hybrid receptors that are (1) capable of binding one or more ligands, and (2) able to respond to the binding by signaling the cell, either directly or indirectly in a manner that is detectable and is distinct from the response of cells not transfected with DNA encoding the hybrid receptor. The response of the receptor to the ligand binding will be detectable by assaying for signaling, such as by a conformational, chemical, or structural change in the receptor (for example, phosphorylation of the receptor), dimerization of the receptor with another molecule, production of a chemical messenger on the surface of or inside of the cell (such as cGMP), immunological detection, growth and/or differentiation, or other assay that is appropriate for the particular hybrid receptor being evaluated.

Detailed Description Text - DETX (43):
Expression of the hybrid receptor polypeptide can be evaluated in a variety

of ways. To measure the level of hybrid receptor protein in the cell, an antibody directed against particular regions of the hybrid receptor may be used in either a Western blot analysis or in an immunoprecipitation analysis. Expression may also be monitored using fluorescence activated cell sorting (FACS). Alternatively, or additionally, bioassays to detect the activity of the hybrid receptor may be used. Here, the ligand or suspected ligand is added to the cell culture along with other reagents as necessary for analyzing receptor activity (for example, .sup.32 P-ATP, .sup.3 H-thymidine, .sup.32 P-GTP, and the like); after an appropriate period of time, the cells are assayed for certain changes that may have occurred in response to ligand binding. Some of these changes may include for example, phosphorylation of the receptor itself or of another protein, production of cGMP or cAMP, or expression of particular genes in the cell. In addition, the rate of host cell proliferation or the rate of host cell death may be a means of measuring hybrid receptor activity.

US-PAT-NO: 5500230

DOCUMENT-IDENTIFIER: US 5500230 A

TITLE: Method for treatment of glaucoma with nitrogen containing guanylate cyclase activators

DATE-ISSUED: March 19, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Nathanson; James A.	Wellesley	MA	N/A	N/A

APPL-NO: 08/ 043979

DATE FILED: April 7, 1993

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation of application Ser. No. 07/702,855, filed Nov. 21, 1990, now abandoned, which is a continuation of application Ser. No. 07/147,324, filed Jan. 22, 1988, now abandoned, which is a continuation-in-part of U.S. application Ser. No. 006,405, filed Jan. 23, 1987 now abandoned which is incorporated by reference herein in its entirety.

US-CL-CURRENT: 424/619, 514/248 , 514/257 , 514/913

ABSTRACT:

The present invention relates to a method of treating cranial fluid volume dysfunctions such as glaucoma in an individual, comprising administering compounds which effect an increase in cyclic GMP at the site of the dysfunction or at the site of synthesis or removal of the accumulating fluid. In particular, the method of treatment involves the topical use of hydralazine, non-organic nitrites or nitroglycerine, and the systematic use of hydralazine or non-organic nitrites.

14 Claims, 25 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 18

---------- KWIC ---------

Detailed Description Text - DETX (12):

Generally, the broken cell preparations are prepared according to the method described in a paper by Nathanson et al., Molecular Pharmacology 20:68 (1981), which is herein incorporated by reference. Choroid epithelial cells are prepared as described in Example 1. To measure activity of atriopeptins, analogues, or agonists thereof, washed particulate cell preparations are prepared by using the pellet obtained following high speed centrifugation. To measure direct activators of guanylate cyclase, the crude broken-cell preparation without centrifugation is used. Guanylate cyclase activity is

measured in appropriate buffer-containing GTP, cofactors, tissue fraction, and the compound to be tested. If necessary, the compounds to be tested are initially solubilized and appropriate solvent controls are run in parallel. The enzyme reaction is initiated by addition of GTP, stopped by heating, and centrifuged. Cyclic GMP can be measured by any test which indicates the presence thereof, typically by the radioimmunoassay as described in Example 6. Normally, the solution mixture contains a phosphodiesterase inhibitor such as theophylline, so as to provide linear measurements with respect to time and enzyme concentration. The determination of the constant K.sub.a, which is the concentration of agonist necessary for half-maximal activation of cyclase activity, is carried out by measuring cyclase activity in the preparation, and plotting the activity (above control activity) versus the semilogarithm of the particular agonist concentration. This is done for a series of increasing concentrations until maximal activity (V.sub.max) is reached. K.sub.a.sup.B, where B is the test compound, is compared with the constant (K.sub.a.sup.ANF) determined in an analogous way using rat atrial natriuretic factor (rANF) as a standard. The ratio K.sub.a.sup.ANF /K.sub.a.sup.B is then an indication of whether the compound (B) is better (ratio greater than 1) or worse (ratio smaller than 1) than rANF. Maximal activation of enzyme activity as a percentage of maximal activation seen in the presence of rANF can be denoted as % V.sub.max.

Detailed Description Text - DETX (38):

Purified secretory epithelial cells were isolated and maintained in tissue culture medium in the absence of any hormones. After 3 hours, rANP was added either alone or in the presence of phosphodiesterase (PDE) inhibitors to a suspension of choroid epithelial cells incubated at 37.degree. C. in artificial CSF. FIG. 2A demonstrates the appearance by phase contrast microscopy of a small group of epithelial cells after isolation and purification. FIG. 2B shows the same cells as in FIG. 2A which have been stained with a rabbit polyclonal antibody (1:150 dilution) to the alpha form of Na,K-ATPase, Sweadner et al., J. Biol. Chem., 260:9016 (1985) followed by second antibody (1:100 dilution). Plasma membrane fluorescence (see also FIG. 2E) was characteristic of choroid epithelium as demonstrated with rhodamine optics. FIG. 2C shows epithelial cells which were also immunostained with mouse monoclonal antibody to DARPP-32, Nestler et al., Science, 225:1357 (1984), followed by second antibody (1:100 dilution). More diffuse staining was found only in epithelium and not in vascular or stromal components of choroid epithelial cells as determined by fluorescein optics. FIG. 2D shows a suspension of choroid epithelial cells incubated at 37.degree. C. in artificial CSF. After exposure for 5 minutes to 1 .mu.M rANP, the cells showed a marked increase in intracellular cyclic GMP content, an effect that was potentiated by the phosphodiesterase inhibitors theophylline (10 mM) and IBMX (0.5 mM). The synergistic effect of phosphodiesterase inhibitors and rANF is demonstrated in FIG. 2D where the cGMP accumulation after treatment with the combination is greater than would be expected from the summation of the effects of phosphodiesterase inhibitors and rANF added individually. For one experiment, the mean.+-.range is shown for duplicate determinations, each assayed in triplicate for cyclic GMP content. In four separate experiments, the degree of stimulation by rANP alone varied from 290% to 1460%. FIG. 2E demonstrates another group of epithelial cells showing bright plasma membrane immunostaining of Na,K-ATPase. FIG. 2F shows the same cells immunostained with antibody to DARPP-32. Fewer than 5% of cells were DARPP-negative.

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FILE 'HOME' ENTERED AT 10:27:38 ON 07 JUN 2004

=> fil .bec
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.21       0.21

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11 FILES IN THE FILE LIST

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         20806 GMP
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                 (CYCLIC(W)GMP)
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                 (CYCLIC(W)GMP)
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           610 CYCLIC GMP
L11        610 CGMP OR CYCLIC GMP

TOTAL FOR ALL FILES
L12     132374 CGMP OR CYCLIC GMP

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FILE 'MEDLINE'
            40 DECTECT?
       1254038 INDICAT?
L13         793 L1 (10A)(DECTECT? OR INDICAT?)

FILE 'SCISEARCH'
            76 DECTECT?
       1184286 INDICAT?
L14         749 L2 (10A)(DECTECT? OR INDICAT?)

FILE 'LIFESCI'
            47 DECTECT?
        379311 INDICAT?
L15         253 L3 (10A)(DECTECT? OR INDICAT?)

FILE 'BIOTECHDS'
             3 DECTECT?
         29094 INDICAT?
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L16           7 L4  (10A)(DECTECT? OR INDICAT?)

FILE 'BIOSIS'
             95 DECTECT?
        1234699 INDICAT?
L17          821 L5  (10A)(DECTECT? OR INDICAT?)

FILE 'EMBASE'
            101 DECTECT?
        1182287 INDICAT?
L18         1087 L6  (10A)(DECTECT? OR INDICAT?)

FILE 'HCAPLUS'
            127 DECTECT?
        1879120 INDICAT?
L19          792 L7  (10A)(DECTECT? OR INDICAT?)

FILE 'NTIS'
             37 DECTECT?
         152028 INDICAT?
L20            2 L8  (10A)(DECTECT? OR INDICAT?)

FILE 'ESBIOBASE'
             14 DECTECT?
         462794 INDICAT?
L21          492 L9  (10A)(DECTECT? OR INDICAT?)

FILE 'BIOTECHNO'
             25 DECTECT?
         331366 INDICAT?
L22          295 L10 (10A)(DECTECT? OR INDICAT?)

FILE 'WPIDS'
             46 DECTECT?
         382162 INDICAT?
L23            9 L11 (10A)(DECTECT? OR INDICAT?)

TOTAL FOR ALL FILES
L24         5300 L12 (10A)(DECTECT? OR INDICAT?)

=> s l24 and (fluorescen? or fret)
FILE 'MEDLINE'
         272223 FLUORESCEN?
            900 FRET
L25           34 L13 AND (FLUORESCEN? OR FRET)

FILE 'SCISEARCH'
         213922 FLUORESCEN?
           1508 FRET
L26           22 L14 AND (FLUORESCEN? OR FRET)

FILE 'LIFESCI'
          52041 FLUORESCEN?
            267 FRET
L27            5 L15 AND (FLUORESCEN? OR FRET)

FILE 'BIOTECHDS'
          13383 FLUORESCEN?
            167 FRET
L28            1 L16 AND (FLUORESCEN? OR FRET)

FILE 'BIOSIS'
         219784 FLUORESCEN?
           1285 FRET
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L29          28 L17 AND (FLUORESCEN? OR FRET)

FILE 'EMBASE'
        152129 FLUORESCEN?
           771 FRET
L30          29 L18 AND (FLUORESCEN? OR FRET)

FILE 'HCAPLUS'
        369744 FLUORESCEN?
          1763 FRET
L31          20 L19 AND (FLUORESCEN? OR FRET)

FILE 'NTIS'
         15440 FLUORESCEN?
            22 FRET
L32           0 L20 AND (FLUORESCEN? OR FRET)

FILE 'ESBIOBASE'
         68509 FLUORESCEN?
           737 FRET
L33          14 L21 AND (FLUORESCEN? OR FRET)

FILE 'BIOTECHNO'
         68737 FLUORESCEN?
           397 FRET
L34          12 L22 AND (FLUORESCEN? OR FRET)

FILE 'WPIDS'
         72511 FLUORESCEN?
          1122 FRET
L35           1 L23 AND (FLUORESCEN? OR FRET)

TOTAL FOR ALL FILES
L36         166 L24 AND (FLUORESCEN? OR FRET)

=> dup rem l36
PROCESSING COMPLETED FOR L36
L37           53 DUP REM L36 (113 DUPLICATES REMOVED)

=> d tot

L37  ANSWER 1 OF 53     MEDLINE on STN                    DUPLICATE 1
TI   Guanylyl cyclase inhibitors NS2028 and ODQ and protein kinase G (PKG)
     inhibitor KT5823 trigger apoptotic DNA fragmentation in immortalized
     uterine epithelial cells: anti-apoptotic effects of basal cGMP/PKG.
SO   Molecular human reproduction, (2003 Dec) 9 (12) 775-83.
     Journal code: 9513710. ISSN: 1360-9947.
AU   Chan Siu Lan; Fiscus Ronald R
AN   2003541266     IN-PROCESS

L37  ANSWER 2 OF 53  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI   Guanylyl cyclase inhibitors NS2028 and ODQ and protein kinase G (PKG)
     inhibitor KT5823 trigger apoptotic DNA-fragmentation in immortalized
     uterine epithelial cells: Anti-apoptotic effects of basal cGMP/PKG.
SO   Molecular Human Reproduction, (November-December 2003) Vol. 9, No. 11-12,
     pp. 775-783. print.
     ISSN: 1360-9947 (ISSN print).
AU   Chan, Siu Lan; Fiscus, Ronald R. [Reprint Author]
AN   2004:65426  BIOSIS

L37  ANSWER 3 OF 53  HCAPLUS  COPYRIGHT 2004 ACS on STN
TI   Cygnets: spatial and temporal analysis of intracellular cGMP
SO   Proceedings of the Western Pharmacology Society (2003), 46, 28-31
     CODEN: PWPSA8; ISSN: 0083-8969
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AU Sawyer, Carolyn L.; Honda, Akira; Dostmann, Wolfgang R. G.
AN 2003:988800 HCAPLUS

L37 ANSWER 4 OF 53 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Ammonia stimulates nitric oxide production but inhibits accumulation of cyclic GMP in a cerebral capillary endothelial cell line.
SO Society for Neuroscience Abstract Viewer and Itinerary Planner, (2003) Vol. 2003, pp. Abstract No. 638.7. http://sfn.scholarone.com. e-file. Meeting Info.: 33rd Annual Meeting of the Society of Neuroscience. New Orleans, LA, USA. November 08-12, 2003. Society of Neuroscience.
AU Zielinska, M. [Reprint Author]; Shanker, G.; Aschner, J. L.; Albrecht, J. [Reprint Author]; Aschner, M.
AN 2004:202538 BIOSIS

L37 ANSWER 5 OF 53 HCAPLUS COPYRIGHT 2004 ACS on STN
TI Circularly permuted **fluorescent** protein indicators for measuring the response of a sensor polypeptide to an environmental parameter
SO U.S. Pat. Appl. Publ., 62 pp., Cont.-in-part of U.S. Ser. No. 316,920.
CODEN: USXXCO
IN Tsien, Roger Y.; Baird, Geoffrey
AN 2002:814909 HCAPLUS
DN 137:334929

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002157120	A1	20021024	US 2001-999745	20011023
US 6699687	B1	20040302	US 1999-316920	19990521
WO 2000071565	A2	20001130	WO 2000-US13684	20000517
WO 2000071565	C2	20020704		

PI
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

L37 ANSWER 6 OF 53 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
TI New cyclic polypeptides useful for preparation of a medicament for treatment of at least one symptom of e.g. Menieres disease.
PI WO 2002079235 A2 20021010 (200321)* EN 96 C07K007-00
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW
AU 2002311556 A1 20021015 (200432) C07K007-00
IN LARSEN, B D; MEIER, E; NEVE, S; PETERSEN, J S; QVORTRUP, K

L37 ANSWER 7 OF 53 MEDLINE on STN DUPLICATE 2
TI Allosteric activation of cGMP-specific, cGMP-binding phosphodiesterase (PDE5) by cGMP.
SO Biochemistry, (2002 Jul 30) 41 (30) 9672-9.
Journal code: 0370623. ISSN: 0006-2960.
AU Okada Daisuke; Asakawa Shigeki
AN 2002400988 MEDLINE

L37 ANSWER 8 OF 53 HCAPLUS COPYRIGHT 2004 ACS on STN
TI Exogenous nitric oxide and endogenous glucose-stimulated β-cell nitric oxide augment insulin release
SO Diabetes (2002), 51(12), 3450-3460

CODEN: DIAEAZ; ISSN: 0012-1797
AU Smukler, Simon R.; Tang, Lan; Wheeler, Michael B.; Salapatek, Anne Marie F.
AN 2002:939485 HCAPLUS
DN 138:199016

L37 ANSWER 9 OF 53 MEDLINE on STN
TI Immunolocalization of multidrug resistance protein 5 in the human genitourinary system.
SO Journal of urology, (2002 May) 167 (5) 2271-5.
Journal code: 0376374. ISSN: 0022-5347.
AU Nies Anne T; Spring Herbert; Thon Walter F; Keppler Dietrich; Jedlitschky Gabriele
AN 2002224613 MEDLINE

L37 ANSWER 10 OF 53 MEDLINE on STN DUPLICATE 3
TI Assay and screening methods for bioactive substances based on cellular signaling pathways.
SO Journal of biotechnology, (2002 Feb) 82 (4) 357-70. Ref: 17
Journal code: 8411927. ISSN: 0168-1656.
AU Umezawa Yoshio
AN 2002255672 MEDLINE

L37 ANSWER 11 OF 53 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 4
TI **cGMP** signaling in smooth muscle cells examined by **fret** -based **indicators**
SO BIOPHYSICAL JOURNAL, (JAN 2002) Vol. 82, No. 1, Part 2, pp. 273A-274A. MA 1323.
Publisher: BIOPHYSICAL SOCIETY, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998 USA.
ISSN: 0006-3495.
AU Sawyer C L (Reprint); Dostmann W R G
AN 2002:161094 SCISEARCH

L37 ANSWER 12 OF 53 HCAPLUS COPYRIGHT 2004 ACS on STN
TI Patch-cram detection of cyclic GMP in intact cells
SO Neuromethods (2002), 35(Patch-Clamp Analysis), 245-263
CODEN: NUROE8; ISSN: 0893-2336
AU Kramer, Richard H.
AN 2002:747136 HCAPLUS
DN 138:350558

L37 ANSWER 13 OF 53 MEDLINE on STN DUPLICATE 5
TI The organic cation transporters rOCT1 and hOCT2 are inhibited by cGMP.
SO Journal of membrane biology, (2002 Oct 1) 189 (3) 237-44.
Journal code: 0211301. ISSN: 0022-2631.
AU Schlatter E; Monnich V; Cetinkaya I; Mehrens T; Ciarimboli G; Hirsch J R; Popp C; Koepsell H
AN 2002635409 MEDLINE

L37 ANSWER 14 OF 53 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Impaired Glycoprotein Ib-IX-Dependent Platelet Activation in Mice Lacking cGMP-Dependent Protein Kinase.
SO Blood, (November 16 2002) Vol. 100, No. 11, pp. Abstract No. 968. print.
Meeting Info.: 44th Annual Meeting of the American Society of Hematology. Philadelphia, PA, USA. December 06-10, 2002. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971.
AU Li, Zhenyu [Reprint Author]; Xi, Xiaodong [Reprint Author]; Gu, Minyi [Reprint Author]; Feil, Robert [Reprint Author]; Ye, Richard [Reprint Author]; Hofmann, Franz [Reprint Author]; Du, Xiaoping [Reprint Author]
AN 2003:335568 BIOSIS

L37 ANSWER 15 OF 53 MEDLINE on STN

TI The vasodilator-stimulated phosphoprotein is regulated by cyclic GMP-dependent protein kinase during neutrophil spreading.
SO Journal of immunology (Baltimore, Md. : 1950), (2001 May 1) 166 (9) 5550-6.
Journal code: 2985117R. ISSN: 0022-1767.
AU Lawrence D W; Pryzwansky K B
AN 2001409985 MEDLINE

L37 ANSWER 16 OF 53 MEDLINE on STN DUPLICATE 6
TI Spatiotemporal dynamics of guanosine 3',5'-cyclic monophosphate revealed by a genetically encoded, **fluorescent** indicator.
SO Proceedings of the National Academy of Sciences of the United States of America, (2001 Feb 27) 98 (5) 2437-42.
Journal code: 7505876. ISSN: 0027-8424.
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AN 2001456582 MEDLINE

L37 ANSWER 17 OF 53 MEDLINE on STN
TI Nitric oxide donor stimulated increase of cyclic GMP in the goldfish retina.
SO Visual neuroscience, (2001 Nov-Dec) 18 (6) 849-56.
Journal code: 8809466. ISSN: 0952-5238.
AU Baldridge W H; Fischer A J
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L37 ANSWER 18 OF 53 MEDLINE on STN DUPLICATE 7
TI ATP and nitric oxide modulate intracellular calcium in isolated pillar cells of the guinea pig cochlea.
SO Journal of the Association for Research in Otolaryngology : JARO, (2001 Dec) 2 (4) 399-407.
Journal code: 100892857. ISSN: 1525-3961.
AU Chung J W; Schacht J
AN 2002102076 MEDLINE

L37 ANSWER 19 OF 53 MEDLINE on STN DUPLICATE 8
TI Nitric oxide modulates Ca(2+) channels in dorsal root ganglion neurons innervating rat urinary bladder.
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Journal code: 0375404. ISSN: 0022-3077.
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AN 2001374801 MEDLINE

L37 ANSWER 20 OF 53 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 9
TI Routine o-glycan characterization in nutritional supplements - a comparison of analytical methods for the monitoring of the bovine kappa-casein macropeptide glycosylation
SO JOURNAL OF CHROMATOGRAPHY A, (21 SEP 2001) Vol. 929, No. 1-2, pp. 151-163.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0021-9673.
AU Tran N T; Daali Y; Cherkaoui S; Taverna M; Neeser J R; Veuthey J L (Reprint)
AN 2001:787471 SCISEARCH

L37 ANSWER 21 OF 53 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 10
TI NO inhibits stretch-induced MAPK activity by cytoskeletal disruption.
SO Journal of Biological Chemistry, (22 Dec 2000) 275/51 (40301-40306).
Refs: 47
ISSN: 0021-9258 CODEN: JBCHA3
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AN 2001032988 EMBASE

L37 ANSWER 22 OF 53 MEDLINE on STN DUPLICATE 11

TI **Fluorescent indicators** for **cyclic GMP** based on **cyclic GMP**-dependent protein kinase Ialpha and green **fluorescent** proteins.
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Journal code: 0370536. ISSN: 0003-2700.
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L37 ANSWER 23 OF 53 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
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SO BIOPHYSICAL JOURNAL, (JAN 2000) Vol. 78, No. 1, Part 2, pp. PO884-PO884.
Publisher: BIOPHYSICAL SOCIETY, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998.
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AN 2000:110426 SCISEARCH

L37 ANSWER 24 OF 53 MEDLINE on STN DUPLICATE 12
TI Cyclic GMP evoked calcium transients in olfactory receptor cell growth cones.
SO Neuroreport, (2000 Mar 20) 11 (4) 677-81.
Journal code: 9100935. ISSN: 0959-4965.
AU Kafitz K W; Leinders-Zufall T; Zufall F; Greer C A
AN 2000218425 MEDLINE

L37 ANSWER 25 OF 53 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 13
TI Characterization of the human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus) oncogene, Kaposin (ORF K12)
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Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 1386-6532.
AU Muralidhar S; Veytsmann G; Chandran B; Ablashi D; Doniger J; Rosenthal L J (Reprint)
AN 2000:299136 SCISEARCH

L37 ANSWER 26 OF 53 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Design of **fluorescent indicators** for **cGMP.**
SO Biophysical Journal, (Jan., 2000) Vol. 78, No. 1 Part 2, pp. 150A. print.
Meeting Info.: 44th Annual Meeting of the Biophysical Society. New Orleans, Louisiana, USA. February 12-16, 2000.
CODEN: BIOJAU. ISSN: 0006-3495.
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L37 ANSWER 27 OF 53 MEDLINE on STN
TI Nitric oxide donors selectively potentiate thrombin-stimulated p70(S6k) activity and morphological changes in Swiss 3T3 cells.
SO Biochemical and biophysical research communications, (1999 Dec 20) 266 (2) 352-60.
Journal code: 0372516. ISSN: 0006-291X.
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AN 2000068770 MEDLINE

L37 ANSWER 28 OF 53 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI Nitric oxide modulates endothelin 1-induced Ca2+ mobilization and cytoskeletal F-actin filaments in human cerebromicrovascular endothelial cells.
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Refs: 28

ISSN: 0271-678X CODEN: JCBMDN
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L37 ANSWER 29 OF 53 MEDLINE on STN DUPLICATE 14
TI The role of cyclic guanylate monophosphate in nitric oxide-induced injury to rat small intestinal epithelial cells.
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Journal code: 0376362. ISSN: 0022-3565.
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L37 ANSWER 30 OF 53 MEDLINE on STN DUPLICATE 15
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Journal code: 0266262. ISSN: 0022-3751.
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AN 1998399980 MEDLINE

L37 ANSWER 31 OF 53 MEDLINE on STN
TI Localization of soluble guanylate cyclase activity in the guinea pig cochlea suggests involvement in regulation of blood flow and supporting cell physiology.
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Journal code: 9815334. ISSN: 0022-1554.
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AN 97459026 MEDLINE

L37 ANSWER 32 OF 53 MEDLINE on STN DUPLICATE 16
TI A cyclic GMP-dependent housekeeping Cl- channel in rabbit gastric parietal cells activated by a vasodilator ecabapide.
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Journal code: 7502536. ISSN: 0007-1188.
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AN 97137156 MEDLINE

L37 ANSWER 33 OF 53 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 17
TI Nitric oxide induces upregulation of Fas and apoptosis in vascular smooth muscle.
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CODEN: HPRTDN. ISSN: 0194-911X.
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AN 1996:183092 BIOSIS

L37 ANSWER 34 OF 53 MEDLINE on STN
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AN 97075021 MEDLINE

L37 ANSWER 35 OF 53 MEDLINE on STN DUPLICATE 18
TI Activation of the nitric oxide/cGMP pathway is required for refilling intracellular Ca2+ stores in a sympathetic neuron cell line.
SO Cell calcium, (1996 May) 19 (5) 399-407.
Journal code: 8006226. ISSN: 0143-4160.
AU Harrington M A; Thompson S H

AN 96385322 MEDLINE

L37 ANSWER 36 OF 53 MEDLINE on STN
TI Distribution of nitric oxide synthase-immunoreactive nerves and identification of the cellular targets of nitric oxide in guinea-pig and human urinary bladder by cGMP immunohistochemistry.
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Journal code: 7605074. ISSN: 0306-4522.
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L37 ANSWER 37 OF 53 MEDLINE on STN DUPLICATE 19
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AU Shah A M; Silverman H S; Griffiths E J; Spurgeon H A; Lakatta E G
AN 95335901 MEDLINE

L37 ANSWER 38 OF 53 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
TI CGMP PREVENTS DELAYED RELAXATION AT REOXYGENATION AFTER BRIEF HYPOXIA IN ISOLATED CARDIAC MYOCYTES
SO AMERICAN JOURNAL OF PHYSIOLOGY-HEART AND CIRCULATORY PHYSIOLOGY, (JUN 1995) Vol. 37, No. 6, pp. H2396-H2404.
ISSN: 0363-6135.
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L37 ANSWER 39 OF 53 MEDLINE on STN DUPLICATE 20
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SO Circulation research, (1994 May) 74 (5) 970-8.
Journal code: 0047103. ISSN: 0009-7330.
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L37 ANSWER 40 OF 53 MEDLINE on STN DUPLICATE 21
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SO Circulation research, (1994 Oct) 75 (4) 659-68.
Journal code: 0047103. ISSN: 0009-7330.
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L37 ANSWER 41 OF 53 MEDLINE on STN
TI Identification and possible localization of cGMP-dependent protein kinase in bovine aortic endothelial cells.
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Journal code: 0372516. ISSN: 0006-291X.
AU MacMillan-Crow L A; Murphy-Ullrich J E; Lincoln T M
AN 94271198 MEDLINE

L37 ANSWER 42 OF 53 MEDLINE on STN DUPLICATE 22
TI Diffusion coefficient of cyclic GMP in salamander rod outer segments estimated with two **fluorescent** probes.
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Journal code: 0370626. ISSN: 0006-3495.
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L37 ANSWER 43 OF 53 MEDLINE on STN DUPLICATE 23
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Journal code: 0401121. ISSN: 0031-1820.
AU Proudfoot L; Kusel J R; Smith H V; Kennedy M W
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L37 ANSWER 44 OF 53 MEDLINE on STN DUPLICATE 24
TI Cyclic GMP increases the rate of the calcium extrusion pump in intact human platelets but has no direct effect on the dense tubular calcium accumulation system.
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Journal code: 0217513. ISSN: 0006-3002.
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L37 ANSWER 45 OF 53 MEDLINE on STN DUPLICATE 25
TI Role of intracellular Ca2+ mobilization in muscarinic and histamine receptor-mediated activation of guanylate cyclase in N1E-115 neuroblastoma cells: assessment of the arachidonic acid release hypothesis.
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Journal code: 0035623. ISSN: 0026-895X.
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AN 90294810 MEDLINE

L37 ANSWER 46 OF 53 MEDLINE on STN
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Journal code: 2985190R. ISSN: 0022-3042.
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AN 88199571 MEDLINE

L37 ANSWER 47 OF 53 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 26
TI ELECTROPHYSIOLOGICAL AND DYE-COUPLING STUDIES ON SECRETORY MYOEPITHELIAL AND DUCT CELLS IN HUMAN ECCRINE SWEAT GLANDS.
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CODEN: JPHYA7. ISSN: 0022-3751.
AU JONES C J [Reprint author]; KEALEY T
AN 1987:421525 BIOSIS

L37 ANSWER 48 OF 53 MEDLINE on STN DUPLICATE 27
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Journal code: 0372516. ISSN: 0006-291X.
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L37 ANSWER 52 OF 53 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 30
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L37 ANSWER 5 OF 53 HCAPLUS COPYRIGHT 2004 ACS on STN
AB The present invention provides an isolated nucleic acid sequence that encodes a **fluorescent** indicator or chimeric construct, the indicator having a sensor polypeptide that is responsive to a chemical, biol., elec. or physiol. parameter, and a **fluorescent** protein moiety, wherein the sensor polypeptide is operatively inserted into the **fluorescent** protein moiety, and wherein the **fluorescence** of the **fluorescent** protein moiety is affected by the responsiveness of the sensor polypeptide. When a sensor polypeptide is inserted into a **fluorescent** protein such as an Aequorea-related **fluorescent** protein (e.g., Green **Fluorescent** Protein (GFP), Yellow **Fluorescent** Protein (YFP), Cyan **Fluorescent** Protein (CFP), or a derivative or mutant thereof) to form a construct, interaction of the sensor polypeptide with a biol., chemical, elec. or physiol. parameter, for example, results in a change in **fluorescence** of the **fluorescent** protein. Such constructs are useful in measuring interactions of a sensor polypeptides with environmental stimuli in vitro or in vivo or in measuring particular characteristics of a cell (e.g., redox potential, intracellular ion concentration). These constructs rely on the responsiveness of a sensor polypeptide inserted within a GFP-sensor-related protein itself to influence the actual **fluorescence** of the fluorophore and not the interaction of tandem **fluorescent** mols. Also provided are circularly permuted **fluorescent** polypeptides and polynucleotides encoding the circularly permuted **fluorescent** polypeptides. In addition, methods of using the **fluorescent** indicators and the circularly permuted **fluorescent** polypeptides are provided.

L37 ANSWER 23 OF 53 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

L37 ANSWER 28 OF 53 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

AB A functional interrelation between nitric oxide (NO), the endothelial-derived vasodilating factor, and endothelin 1 (ET-1), the potent vasoconstrictive peptide, was investigated in microvascular endothelium of human brain. Nor-1 dose-dependently decreased the ET-1-stimulated mobilization of Ca2+. This response was mimicked with cGMP and abrogated by inhibitors of guanylyl cyclase or **cGMP**-dependent protein kinase G. These findings **indicate** that NO and ET-1 interactions involved in modulation of intracellular Ca2+ are mediated by cGMP/protein kinase G. In addition, Nor- 1-mediated effects were associated with rearrangements of cytoskeleton F- actin filaments. The results suggest mechanisms by which NO-ET-1 interactions may contribute to regulation of microvascular function.

L37 ANSWER 29 OF 53 MEDLINE on STN DUPLICATE 14

AB In our study we have examined the importance of cyclic guanylate monophosphate (cGMP) in NO-mediated intestinal cellular damage. Epithelial cells were harvested from a 20-cm segment of rat proximal small intestine by dispersion using citrate and ethylenediaminetetraacetic acid. Cell viability was assessed by trypan blue dye exclusion. Incubation of cells with the nitric oxide donors, S-nitroso-N-acetyl penicillamine (SNAP) or sodium nitroprusside (SNP) (10-1000 microM) produced a concentration-dependent increase in cell injury and an increase in cellular cGMP formation as determined by immunoassay. In addition, cell injury was also increased by treatment of cells with the cell permeable analogue, dibutryryl cGMP (db cGMP; 0.1-2.0 mM). Suppression of cellular cGMP production by incubating cells with the guanylate cyclase inhibitor LY83583 (5-20 microM) attenuated the damaging actions of SNAP or SNP. However, LY83583 treatment did not reduce ethanol-mediated (10% v/v) cell injury. Furthermore the cytotoxic actions of SNAP or SNP were enhanced by preincubation of cells with the selective cGMP phosphodiesterase inhibitor, zaprinast (10 mM). The damaging actions of SNAP, SNP and db cGMP were reduced by treating cells with superoxide dismutase (100 U/ml). Similarly SNAP, SNP and db cGMP treatments resulted in an increase in the in vitro production of reactive oxygen metabolites as assessed by the **fluorescent** probe 2'7' dichlorofluoresein diacetate. These findings **indicate** that **cGMP** mediates intestinal cell injury in response to high levels of nitric oxide as produced by the nitric oxide donors, SNAP and SNP. Furthermore these data suggest that the cGMP-induced damage to intestinal epithelial cells involves the generation of reactive oxidants.

L37 ANSWER 30 OF 53 MEDLINE on STN DUPLICATE 15

AB 1. Inhibition of inositol 1,4,5-trisphosphate (IP3) receptor-mediated Ca2+ release by cGMP was examined in intact rat megakaryocytes, by using a combination of single cell **fluorescence** microscopy to monitor intracellular free calcium ([Ca2+]i) and flash photolysis of caged second messengers. 2. Sodium nitroprusside (SNP), a nitric oxide (NO) donor, and the hydrolysis-resistant cGMP analogue 8-(4-chlorophenylthio)guanosine 3',5'-cyclic monophosphate (pCPT-cGMP) inhibited Ca2+ release induced by photolysis of caged IP3. Neither of them affected the rate of Ca2+ removal from the cytoplasm following photolysis of caged Ca2+. 3. Photolysis of the caged NO donor 3-morpholinosydnonimine (SIN-1) during agonist-induced [Ca2+]i oscillations inhibited Ca2+ release without affecting the rate of Ca2+ uptake and/or extrusion. 4. We conclude that the inhibition of IP3-induced Ca2+ release is the principal mechanism of NO-cGMP-dependent inhibition of [Ca2+]i mobilization. 5. IPG, a specific peptide inhibitor of cGMP-dependent protein kinase (**cGMP**-PK), blocked the inhibitory effect of pCPT-**cGMP**, **indicating** that the inhibition of IP3-induced Ca2+ release by pCPT-**cGMP** is mediated by cGMP-PK. However, the simultaneous application of both IPG and IP20, a specific peptide inhibitor of cAMP-dependent protein kinase

(cAMP-PK), was required to block the inhibitory effect of SNP. These data strongly suggest that NO-cGMP-dependent inhibition of [Ca2+]i mobilization is mediated via the activation of both cGMP-PK and cAMP-PK.

L37 ANSWER 31 OF 53 MEDLINE on STN
AB Although the nitric oxide/cGMP pathway has many important roles in biology, studies of this system in the mammalian cochlea have focused on the first enzyme in the pathway, nitric oxide synthase (NOS). However, characterization of the NO receptor, soluble guanylate cyclase (sGC), is crucial to determine the cells targeted by NO and to develop rational hypotheses of the function of this pathway in auditory processing. In this study we characterized guinea pig cochlear sGC by determining its enzymatic activity and cellular localization. In cytosolic fractions of auditory nerve, lateral wall tissues, and cochlear neuroepithelium, addition of NO donors resulted in three- to 15-fold increases in cGMP formation. NO-stimulated sGC activity was not detected in particulate fractions. We also localized cochlear sGC activity through immunocytochemical detection of NO-stimulated cGMP. sGC activity was detected in Hensen's and Deiters' cells of the organ of Corti, as well as in vascular pericytes surrounding small capillaries in the lateral wall tissues and sensory neuroepithelium. sGC activity was not observed in sensory cells. Using NADPH-diaphorase histochemistry, NOS was localized to pillar cells and nerve fibers underlying hair cells. These results **indicate** that the NO/**cGMP** pathway may influence diverse elements of the auditory system, including cochlear blood flow and supporting cell physiology.

L37 ANSWER 32 OF 53 MEDLINE on STN DUPLICATE 16
AB 1. The membrane potential of rabbit gastric parietal cells is dominated by a Cl- channel with a subpicosiemens single channel conductance in the basolateral membrane. The effects of 3-[[[2-(3,4-dimethoxyphenyl)ethyl]carbamoyl]amino-N-methylbenzamide++ + (DQ-2511: ecabapide), a vasodilator, on the opening of this Cl-1 channel, the cyclic GMP content and the intracellular free Ca2+ concentration ([Ca2+]i) of parietal cells were investigated by whole-cell patch-clamp technique, enzyme immunoassay and Fura 2-**fluorescence** measurement. 2.Ecabapide stimulated the opening of the Cl-1 channel as determined by the reversal potential. This stimulation was concentration-dependent, and its EC50 value was 0.2 microM. Both the basal and ecabapide-induced openings of the channel were inhibited by 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB, 500 microM), a Cl- channel blocker. Another Cl- channel blocker, niflumic acid (500 microM) was much less effective. 3. The power spectra of the currents before and after the addition of ecabapide (10 microM) were analysed. Both spectra contained only one Lorentzian (1/f2) component. 4. 6-Anilino-5,8-quinolinedione (LY83583; 5 microM) which prevents activation of soluble guanylate cyclase, significantly inhibited both the basal and ecabapide (10 microM)-induced openings of the Cl-channel. 5. Ecabapide (0.01-100 microM) concentration-dependently elevated the cyclic GMP content in the parietal cell-rich suspension. The EC50 value was 0.2 microM. 6. In single Fura 2-loaded parietal cells, ecabapide (10-100 microM) did not increase [Ca2+]i. 7. These results **indicate** that ecabapide stimulates an intracellular production of **cyclic GMP** in the parietal cell without increasing [Ca2+]i, and leads to an activation of the housekeeping Cl- channel.

L37 ANSWER 33 OF 53 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 17
AB Interleukin-1 induced a time-dependent release of high levels of nitric oxide from rat vascular smooth muscle cells up to 96 hours. A time-dependent release of lactate dehydrogenase was also induced by Interleukin-1 from 72 to 96 hours after its stimulation. In situ nick end-labeling assay revealed that incubation for 48 hours with interleukin-1 induced a positive staining of fragmented nuclei. However, N-G-monomethyl-L-arginine, an inhibitor of nitric oxide synthase,

inhibited both lactate dehydrogenase release and DNA fragmentation induced by interleukin-1. Furthermore, sodium nitroprusside, a nitric oxide donor, also induced lactate dehydrogenase release and DNA fragmentation. **Fluorescent** staining of DNA revealed patches of irregularly dispersed, brightly staining, and condensed chromatin in rat vascular smooth muscle cells treated with sodium nitroprusside. Flow cytometric analysis with monoclonal antibody against human Fas revealed that expression of Fas was upregulated by sodium nitroprusside in human vascular smooth muscle cells. Methylene blue, an inhibitor of soluble guanylate cyclase, did not affect sodium nitroprusside-induced upregulation of Fas. Furthermore, 8-bromoguanosine 3':5'-cyclic monophosphate, an analogue of **cGMP**, did not upregulate Fas expression, These findings **indicate** that nitric oxide released from vascular smooth muscle cells may induce apoptosis in vascular smooth muscle cells themselves and also induces upregulation of Fas via a cGMP-independent mechanism. Thus, nitric oxide could trigger the remodeling of atherosclerotic plaques.

L37 ANSWER 34 OF 53 MEDLINE on STN
AB The hormone-induced depletion of cellular Ca stores provides a signal for the Ca2+ influx into electrically non-excitable cells; however, the underlying molecular mechanisms remain elusive. Therefore, we analyzed bradykinin-activated Ca2+ influx into human foreskin fibroblast cells, HF-15, by fura-2 and 45Ca labeling to discriminate between Ca2+ influx into the fura-sensitive compartment and Ca uptake into fura-insensitive Ca stores. Bradykinin-activated Ca2+ influx into the fura-sensitive compartment was blocked by inhibitors of NO synthases. These inhibitors also suppressed bradykinin-activated increases in **cGMP**, **indicating** that the NO-dependent increase in **cGMP** is involved in the activation of the Ca2+ influx into the fura-sensitive compartment. The cGMP-dependent kinase inhibitors KT5823 and Rp-8-(parachlorophenylthio)-cGMP (Rp-8-pCPT-cGMPS) blocked bradykinin-activated Ca2+ influx into the fura-sensitive compartment, suggesting that a cGMP-dependent kinase step participates in the activation of this Ca2+ influx pathway. In addition to the NO/cGMP-mediated Ca2+ influx into the fura-sensitive compartment, bradykinin enhanced 45Ca uptake into Ca stores that were not accessible to fura-2. This enhanced 45Ca uptake was insensitive to blockers of the NO/**cGMP** pathway, **indicating** that the 45Ca uptake pathway is distinct from the NO-dependent Ca2+ influx into the fura-sensitive compartment. Furthermore, bradykinin enhanced 45Ca uptake into proliferating but not into quiescent HF-15 fibroblasts. Hence, bradykinin stimulates two distinct Ca2+ influx pathways in HF-15 cells, (a) Ca2+ influx into the fura-sensitive compartment which is NO/cGMP-dependent and (b) Ca uptake into Ca stores which bypasses the cytoplasm, which is NO insensitive and which is linked to cell proliferation.

L37 ANSWER 35 OF 53 MEDLINE on STN DUPLICATE 18
AB Fura-2 **fluorescence** imaging was used to measure changes in intracellular Ca2+ concentration in individual N1E-115 neuroblastoma cells during repeated activation of M1 muscarinic receptors with carbachol. Ca2+ transients could be elicited repeatedly at 4 min intervals with little decrement as long as external Ca2+ was present. When the cells were bathed in Ca(2+)-free saline, however, the response amplitude decreased rapidly in a use-dependent fashion, indicating that external Ca2+, and presumably Ca2+ influx, is required for refilling Ca2+ stores during the interval between trials. The response amplitude also decreased during repeated stimulation in cells treated with the NO-synthase inhibitor L-NMMA or with the guanylyl cyclase inhibitor LY-83583 even when Ca2+ was present. Application of the membrane permeable cGMP analog 8-Br-cGMP reversed the effect of L-NMMA and promoted refilling in the continued presence of NO-synthase inhibitor. These results **indicate** that activation of the NO/**cGMP** pathway is necessary for refilling Ca2+ stores during muscarinic signaling. Evidence

is also presented suggesting that the NO/cGMP pathway is involved in long term modulation of the content of Ca2+ stores.

L37 ANSWER 36 OF 53 MEDLINE on STN
AB The distribution of nerves with the potential to synthesize nitric oxide was examined within the urinary bladder and proximal urethra of humans and guinea-pigs, using an antibody to nitric oxide synthase. Further experiments identified cells in which cGMP-immunoreactivity was induced following exposure to the nitric oxide donor, sodium nitroprusside. These cells represent the potential physiological targets of neuronally released nitric oxide, since activation of soluble guanylate cyclase, and a consequent rise in intracellular cGMP, mediate many of the effects of this transmitter. Nitric oxide synthase-immunoreactivity was widely distributed in the lower urinary tract. In guinea-pigs, 50-68% of all intrinsic vesical neurons expressed nitric oxide synthase-immunoreactivity, while in humans 72-96% of neurons in the wall of the bladder contained nitric oxide synthase. In both humans and guinea-pigs, varicose nitric oxide synthase-immunoreactive nerve terminals provided a moderate innervation to the detrusor muscle of the bladder body, and a denser innervation to the urethral muscle. Immunoreactive nerves also projected to the subepithelium and around blood vessels, but were rarely observed encircling intramural vesical ganglia. Following stimulation with sodium nitroprusside, smooth muscle cells of the urethra expressed strong cGMP-immunoreactivity, but detrusor muscle cells remained uniformly negative. Although the detrusor muscle fibres did not express cGMP, numerous interstitial cells throughout the bladder body demonstrated an intense induction of cGMP-immunoreactivity by sodium nitroprusside. These cells had long dendritic processes extending parallel to the smooth muscle fibres, and contained vimentin, an intermediate filament expressed by cells of mesenchymal origin. Other cell types in which sodium nitroprusside exposure induced cGMP-immunoreactivity were the uroepithelial cells, vascular smooth muscle cells and pericytes, and a small number of varicose nerve terminals. In the guinea-pig, a minor proportion (less than 10%) of intrinsic neurons in the wall of the bladder also expressed cGMP. No intrinsic neurons were observed in specimens of human bladder processed for cGMP immunohistochemistry. The results provide anatomical evidence that nitric oxide may function as a neurotransmitter in the lower urinary tract. Although nerves with the capacity to produce nitric oxide supply both the detrusor muscle and the urethra, distinct regional differences exist in the effects of nitric oxide on the induction of cGMP. If the nitric oxide-mediated induction of **cGMP** is a reliable **indicator** of the physiological responsiveness of a cell to nitric oxide, then smooth muscle cells appear to be the predominant targets of nitric oxide in the urethra, while in the bladder body, interstitial cells may serve this role. These findings support previous studies which have implicated nitric oxide as an inhibitory transmitter involved in the relaxation of the bladder neck. Our experiments further indicate that a number of cell types within the lower urinary tract could potentially mediate the effects of endogenously released nitric oxide.

L37 ANSWER 37 OF 53 MEDLINE on STN DUPLICATE 19
AB Previous studies in isolated cardiac myocytes suggest that impaired relaxation during reoxygenation after brief hypoxia results from abnormal Ca(2+)-myofilament interaction. Recent studies **indicate** that guanosine 3',5'-cyclic monophosphate (**cGMP**)-elevating interventions selectively enhance myocardial relaxation. We investigated the effect of 8-bromoguanosine 3',5'-cyclic monophosphate (8-BrcGMP) on posthypoxic relaxation in single rat myocytes, with simultaneous measurement of contraction and intracellular Ca2+ (indo 1 **fluorescence**). In control myocytes (n = 11), reoxygenation after 10 min of hypoxia markedly prolonged time to peak shortening (+36.5 +/- 4.2%) and half-relaxation time (+75.7 +/- 11.3% cf. normoxic values; both $P < 0.001$) and reduced diastolic length but did not change cytosolic Ca2+.

Under normoxic conditions, 50 microM 8-BrcGMP slightly reduced time to peak shortening and half-relaxation time and increased diastolic length but did not alter cytosolic Ca2+. In the presence of 8-BrcGMP, there was no posthypoxic delay in twitch relaxation nor was there a decrease in diastolic length (half-relaxation time -5.8 +/- 3.3% cf. normoxic values; $P < 0.05$ cf. control group; n = 11). Cytosolic Ca2+ remained unaltered. Thus, 8-BrcGMP fully prevents impaired posthypoxic relaxation in isolated cardiac myocytes, probably by altering Ca(2+)-myofilament interaction.

L37 ANSWER 38 OF 53 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
AB Previous studies in isolated cardiac myocytes suggest that impaired relaxation during reoxygenation after brief hypoxia results from abnormal Ca2+-myofilament interaction. Recent studies **indicate** that guanosine 3',5'-cyclic monophosphate (**cGMP**)-elevating interventions selectively enhance myocardial relaxation. We investigated the effect of 8-bromoguanosine 3',5'-cyclic monophosphate (8-BrcGMP) on posthypoxic relaxation in single rat myocytes, with simultaneous measurement of contraction and intracellular Ca2+ (indo 1 **fluorescence**). In control myocytes (n = 11), reoxygenation after 10 min of hypoxia markedly prolonged time to peak shortening (+36.5 +/- 4.2%) and half-relaxation time (+75.7 +/- 11.3% cf. normoxic values; both $P < 0.001$) and reduced diastolic length but did not change cytosolic Ca2+. Under normoxic conditions, 50 mu M 8-BrcGMP slightly reduced time to peak shortening and half-relaxation time and increased diastolic length but did not alter cytosolic Ca2+. In the presence of 8-BrcGMP, there was no posthypoxic delay in twitch relaxation nor was there a decrease in diastolic length (half-relaxation time -5.8 +/- 3.3% cf. normoxic values; $P < 0.05$ cf. control group; n = 11). Cytosolic Ca2+ remained unaltered. Thus, 8-BrcGMP fully prevents impaired posthypoxic relaxation in isolated cardiac myocytes, probably by altering Ca2+-myofilament interaction.

L37 ANSWER 39 OF 53 MEDLINE on STN DUPLICATE 20
AB The role of cGMP in myocardial contraction is not established. Recent reports suggest that nitric oxide, released by endothelial cells or within myocytes, modifies myocardial contraction by raising cGMP. We studied the effects of 8-bromo-cGMP (8bcGMP, 50 mumol/L) on contraction (cell shortening) and simultaneous intracellular Ca2+ transients (indo 1 **fluorescence** ratio) in intact adult rat ventricular myocytes (0.5 Hz and 25 degrees C) 8bcGMP reduced myocyte twitch amplitude and time to peak shortening (-19.6 +/- 4.2% and -17.6 +/- 1.3%, respectively) and increased steady-state diastolic cell length (+0.6 +/- 0.1 microns, mean +/- SEM, n = 8; all $P < .05$) but had no effect on shortening velocity, systolic or diastolic **fluorescence** ratio, or time to peak **fluorescence** ratio (all P = NS). In 7 of 13 myocytes, this negative inotropic effect was preceded by a transient positive inotropic effect, with small increases in twitch amplitude, shortening velocity, and cytosolic Ca2+ transient. Analysis of 8bcGMP effects on both the dynamic and steady-state relation between cell shortening and intracellular Ca2+ (during twitch contraction and tetanic contraction, respectively) indicated reduction in the myofilament response to Ca2+ in all cases. These 8bcGMP effects were inhibited by KT5823 (1 mumol/L), an inhibitor of cGMP-dependent protein kinase, or by the presence of isoproterenol (3 nmol/L). 8bcGMP had no effect on cytosolic pH in cells (n = 4) loaded with the **fluorescent** probe carboxyseminaphthorhodafluor-1. These data **indicate** that **cGMP** may modulate myocardial relaxation and diastolic tone by reducing the relative myofilament response to Ca2+, probably via cGMP-dependent protein kinase.

L37 ANSWER 40 OF 53 MEDLINE on STN DUPLICATE 21
AB Altered release of endothelium-derived relaxing factor/nitric oxide (EDRF/NO) has been proposed as a final common pathway underlying the abnormal vasodilator responses to gram-negative lipopolysaccharide (endotoxin). However, mechanisms responsible for lipopolysaccharide-induced changes in EDRF/NO release from endothelial cells have not been

clarified. We evaluated direct effects of Escherichia coli endotoxin on agonist-stimulated cytosolic Ca2+ mobilization and NO biosynthesis in cultured bovine and porcine aortic endothelial cells (ECs). Two methods were used to assay for NO: (1) analysis of NO-induced endothelial levels of **cGMP** as a biological **indicator** of NO generation and (2) direct quantitative measurement of NO release (chemiluminescence method). Cytosolic free Ca2+ ([Ca2+]i) was evaluated using fura 2 **fluorescence** methodology (340/380-nm ratio excitation and 500-nm emission). Incubation of ECs with endotoxin (0.5 microgram/mL, 1 hour plus 1-hour wash) significantly inhibited bradykinin (100 nmol/L)- and ADP (10 mumol/L)-mediated increases in endothelial cell cGMP to 37% and 22% of control responses, respectively. In contrast, endotoxin failed to inhibit the increase in cGMP produced by the non-receptor-dependent Ca2+ ionophore A23187 (1 mumol/L) or sodium nitroprusside (1 mmol/L). Similarly, incubation with endotoxin inhibited ADP-stimulated increases in NO release and EDRF bioactivity to 55% and 56% of control values, respectively, but did not affect A23187-stimulated increases in NO release or EDRF bioactivity. Endotoxin produced significant decreases in both transient and sustained [Ca2+]i responses of ECs to bradykinin and ADP. For example, the initial rapid increase in bovine EC [Ca2+]i in response to bradykinin was reduced to 31% of the initial increases in control cells, and the secondary plateau phase was reduced to only 3% of respective control responses. Concentration-response relation to endotoxin (10(-3)) to 10(0) micrograms/mL) indicated high correlation and similar IC50 values (0.025 and 0.021 micrograms/mL, respectively) for inhibitory effects on cGMP and [Ca2+]i. Endotoxin had no effect on inositol trisphosphate formation ([3H]myo-inositol incorporation) and intracellular Ca2+ release ([Ca2+]i responses in Ca(2+)-free medium) induced by bradykinin. However, agonist-stimulated Mn2+ quenching (index of Ca2+ influx) was significantly attenuated by endotoxin treatment. These studies demonstrate that endotoxin directly decreases agonist (bradykinin and ADP)-mediated biosynthesis and release of EDRF/NO from ECs. These effects can be explained by altered [Ca2+]i mobilization mechanisms, which in turn produce subsequent decreases in activity of the Ca(2+)-calmodulin-dependent constitutive isoform of NO synthase and, ultimately, impairment of agonist-mediated NO release and endothelium-dependent vasodilation.

L37 ANSWER 41 OF 53 MEDLINE on STN

AB Endothelial-derived nitric oxide (NO) is an important intercellular messenger. Although endothelial cells contain both nitric oxide synthase and soluble guanylate cyclase, the nature of receptor proteins for cGMP is uncertain. Based on previous work in vascular smooth muscle cells which **indicates** that the **cGMP**-dependent protein kinase (cGK) is partially associated with the cytoskeleton, we determined that cGK was present in non-cytosolic fractions of endothelial cells. The data reveal that cGK is found only in Triton-soluble extracts of particulate fractions from bovine aortic endothelial cells and provide the first evidence for the existence of cGK in this cell type based on immunoreactivity, immunofluorescence microscopy and phosphotransferase activity. The limited distribution of endothelial cell cGK may explain why this kinase has not been heretofore identified in endothelial cells.

L37 ANSWER 42 OF 53 MEDLINE on STN DUPLICATE 22

AB Experiments have demonstrated that single photoisomerizations in amphibian and primate rods can cause the suppression of 3-5% of the dark circulating current at the response peak (Baylor, D. A., T. D. Lamb, and K. W. Yau. 1979. J. Physiol. (Lond.). 288:613-634; Baylor, D. A., B. J. Nunn, and J. L. Schnapf. 1984. J. Physiol. (Lond.). 357:575-607). These results **indicate** that the change in [**cGMP**] effected by a single isomerization must spread longitudinally over at least the corresponding fractional length of the outer segment. The effective longitudinal diffusion coefficient, Dx, of cGMP is thus an important determinant of rod sensitivity. We report here measurements of the effective longitudinal diffusion coefficients, Dx, of two

fluorescently labeled molecules: 5/6-carboxyfluorescein and 8-(fluoresceinyl)thioguanosine 3',5'-cyclic monophosphate, introduced into detached outer segments via whole-cell patch electrodes. For these compounds, the average time for equilibration of the entire outer segment with the patch pipette was approximately 6 min. **Fluorescence** images of rods were analyzed with a one-dimensional diffusion model that included limitations on transfer between the electrode and outer segment and the effects of intracellular binding of the dyes. The analyses yielded estimates of Dx of 1.9 and 1.0 microns 2.s-1 for the two dyes. It is shown that these results place an upper limit on Dx for cGMP of 11 microns2.s-1. The actual value of Dx for cGMP in the rod will depend on the degree of intracellular binding of cGMP. Estimates of the effective buffering power for cGMP in the rod at rest range from two to six (Lamb and Pugh, 1992; Cote and Brunnock, 1993). When combined with these estimates, our results predict that for cGMP itself, Dx falls within the range of 1.4-5.5 microns 2.s-1.

L37 ANSWER 43 OF 53 MEDLINE on STN DUPLICATE 23
AB Previous work has shown that the surface of infective larvae of parasitic nematodes will not bind the **fluorescent** lipid analogue 5-N-(octadecanoyl)aminofluorescein (AF18) until after exposure of the parasite to mammalian tissue-culture conditions. In this study, culture media which are permissive or non-permissive for the acquisition of lipophilicity for AF18 were altered in order to examine possible stimuli involved. This showed that external alkaline pH and high sodium ion concentration were highly stimulatory. The internal signalling pathways which may be involved in the surface alteration were then examined using agents which are known to affect intracellular signalling in mammalian cells. The results **indicated** that elevation of **cGMP** levels was stimulatory whereas inhibition of a putative Na+/H+ antiporter or calcium mobilization was inhibitory, and it is argued that high intracellular levels of cAMP may be inhibitory. Whilst the precise effects of the agents used on nematode cells remain to be established, these results provide a framework for the examination of the processes involved in the modification of the nematode surface which takes place immediately after the infection event.

L37 ANSWER 44 OF 53 MEDLINE on STN DUPLICATE 24
AB Sodium nitroprusside (SNP) and other agents that elevate cGMP levels are known to inhibit the aggregation of human platelets. Published data suggest that cGMP attenuation of agonist-induced Ca2+ transients is involved in this effect. The present study shows that elevation of cGMP increases the rate of the Ca2+ extrusion pump located in the plasma membrane (PM) but does not have a direct effect on the Ca2+ accumulating pump of the dense tubules (DT). The study verifies that SNP can specifically elevate the cGMP level in the platelet. The kinetics of the Ca2+ extrusion system were studied in situ in platelets overloaded with the cytoplasmic Ca2+ indicator quin2 according to a published protocol developed in this laboratory. Elevation of cGMP by means of (10 microM) SNP increased the Vm of the Ca(2+)-ATPase pump by 63%, without affecting its Km (66-80 nM) or Hill coefficient (1.6-1.8). Dibutyryl-cGMP (Bt2-cGMP), preincubated for 45 min at 1 mM, increased the Vm by a factor of 2.2 +/- 0.4. The experiments did not give any **indication** that SNP or Bt2-**cGMP** change the rate of the Na+/Ca2+ exchanger which makes a minor contribution to Ca2+ extrusion in the studied [Ca2+]cyt range. The rate constant for passive leakage of Ca2+ across the PM was increased by 32 +/- 4% by SNP and 90 +/- 34% by Bt2-cGMP. The net result is that the free Ca2+ in the cytoplasm ([Ca2+]cyt) at 'rest' is lowered from control values of 112 nM to 89 nM or 80 nM, respectively. The kinetics of Ca2+ uptake by the dense tubules were determined in situ using the **fluorescence** of chlorotetracycline (CTC) according to protocols developed in this laboratory. Analysis showed that SNP and Bt2-cGMP had no effect on the Vm or Km of the dense tubular pump, and did not affect the rate constant for passive leakage. The agents did decrease

resting [Ca2+]dt by 25% or 30%, respectively, but this result can be explained purely in terms of the reduced [Ca2+]cyt. The effects of cGMP (vs. cAMP) on the PM and DT pumps are closely correlated with reported effects of cGMP/cAMP induced phosphorylation of a protein of the molecular weight of the PM pump and a 22 kDa activator of the DT pump. Cyclic AMP increases the rate of both the PM and the DT pumps, whereas cGMP increases the rate of the PM pump only.(ABSTRACT TRUNCATED AT 400 WORDS)

L37 ANSWER 45 OF 53 MEDLINE on STN DUPLICATE 25
AB This study evaluates the role of intracellular levels of Ca2+ [Ca2+]i in cyclic GMP formation mediated by muscarinic and histamine receptors in the mouse neuroblastoma clone N1E-115. Muscarinic agonists activated the turnover of phosphoinositides with a relative maximal response similar to that observed previously for cyclic GMP formation. Carbamylcholine induced a transient increase in inositol trisphosphate with a time course similar to that of cyclic GMP formation. In cells loaded with the **fluorescent** Ca2+ probe fura-2/acetoxymethyl ester, carbamylcholine as well as histamine induced a rapid and transient rise in [Ca2+]i. The time course of the changes in [Ca2+]i induced by agonists as well as by ionomycin closely paralleled that of cyclic GMP formation. Chelation of [Ca2+]i by loading of N1E-115 cells with quin 2/acetoxymethyl ester inhibited cyclic GMP formation induced by agonists in a dose-dependent manner. When cyclic GMP formation induced by agonists was assayed after the cells were exposed to 3 mM ethylene glycol bis(beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) for 2 min, the formation of cyclic GMP was not inhibited significantly; however, it was completely abolished after 30-min exposure to EGTA. Treatment of cells with phospholipase A2 had no effect on resting [Ca2+]i and only slightly increased cyclic GMP formation, in spite of the induction of a marked release of [3H]arachidonate. Moreover, the formation of cyclic GMP induced by ionomycin was inhibited by the addition of phospholipase A2. Melittin contaminated with phospholipase A2 activity induced a rapid and sustained increase in cyclic GMP formation, as well as unesterified [3H]arachidonate release. However, after inactivation of the phospholipase A2 activity of melittin, its ability to stimulate cyclic GMP formation was enhanced. Our data **indicate** that receptor agonists stimulate **cyclic GMP** formation in N1E-115 cells by activating the formation of inositol trisphosphate, which is followed by the release of Ca2+ from intracellular stores. The evidence obtained does not support a major role for arachidonate release in receptor-mediated activation of guanylate cyclase. Conversely, it is consistent with an inhibitory role for arachidonic acid or its metabolites in this process.

L37 ANSWER 46 OF 53 MEDLINE on STN
AB Various prostaglandins (PGs) (10 nM-30 microM) were added to NG108-15 cells in culture, and changes in the levels of intracellular cyclic GMP and Ca2+ were investigated. Exposure of the cells to PGF2 alpha, PGD2, and PGE2 (10 microM) transiently increased the cyclic GMP content 7.5-, 3.9-, and 3.1-fold, respectively. Furthermore, the increased levels of cyclic GMP correlated well with the rise in cytosolic free Ca2+ concentrations induced by the PGs. Other PGs (10 microM), including metabolites and synthetic analogs, which had no effect on intracellular Ca2+, failed to increase the cyclic GMP content in the cells. When extracellular Ca2+ was depleted from the culture medium, the PG-induced increase in cyclic GMP level was almost completely abolished. In addition, treatment of the cells with quin 2 tetraacetoxymethyl ester dose-dependently inhibited the PG-induced cyclic GMP formation. The increase in cyclic GMP content caused by treatment of the cells with a high K+ level (50 mM) was completely blocked by voltage-dependent Ca2+ entry blockers, such as verapamil (10 microM), nifedipine (1 microM), and diltiazem (100 microM); however, the PG (10 microM)-induced increase in cyclic GMP content was not affected by such Ca2+ entry blockers. These findings **indicate** that PG-induced **cyclic GMP**

formation may require the rise in intracellular Ca2+ level and that the voltage-dependent Ca2+ channels may not be involved in the PG-induced rise in Ca2+ content.

L37 ANSWER 47 OF 53 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 26

AB 1. Electrophysiological properties and dye-coupling status of secretory, myoepithelial and coiled duct cells in isolated human eccrine sweat glands have been assessed by single-micro-electrode studies and intracellular micro-iontophoresis of the **fluorescent** naphthalimide dye Lucifer Yellow CH (molecular weight 457). Treated glands were embedded in LKB HistoResin and examined by transmission **fluorescence** microscopy, first as wholemounts and then as 5 μm serial sections. Sections positive for Lucifer Yellow were photographed and then stained with Toluidine Blue for observation by conventional microscopy to confirm the site of penetration. 2. Out of forty-five successful micro-iontophoreses, there were confirmed in secretory cells, twelve in myoepithelial cells and thirty in cells of the coiled duct wall. The latter were identified as the most penetrable in the coiled part of the isolated human eccrine sweat gland. 3. Of the three secretory cells labelled (resting potentials -40,-52 and -63 mV), all demonstrated dye coupling to neighbouring secretory cells although in one case this was found to be selective. Not every secretory cell was involved in coupling. No **fluorescent** label spread to the myoepithelial cells which form a network on the basal surface of the secretory tubule. 4. When myoepithelial cells were penetrated, they demonstrated dye coupling to neighbouring myoepithelial cells but not to secretory cells with which they also made contact. Basal resting potentials of -35 to -65 mV were recorded (mean = -52 mV. S.E. of mean = ± 2.4 mV, n = 12) and, in eight out of the twelve cells penetrated and labelled, spontaneous depolarizing transients were also observed whose amplitude but not frequency increased with increasing membrane polarization. Administration of acetylcholine to produce a final concentration of 10-6 to 10-7 M produced either depolarization or micro-electrode dislodgement. 5. Of the thirty cells labelled in the coiled duct, twenty-six showed obvious dye spread to neighbouring cells in both layers of the wall. There was no relation between dye-coupling status and basal resting potentials which lay in the range -40 to -82 mV (mean = -60 mV, S.E. of mean = ± 2.4 mV, n = 30). Repeated doses of acetylcholine produced either no response from cells in this range or depolarization in cells with resting potentials more negative than -70 mV and hyperpolarization in cells with resting potentials more positive than -70 mV. In addition, there was a biphasic response, depolarization followed by hyperpolarization in a cell of resting potential -67 mV. 6. Acetylcholine induced a rise in the cyclic GMP content of whole sweat glands, a characteristic muscarinic response. In contrast, there was no acetylcholine-induced rise in the **cyclic GMP** content of isolated ducts **indicating** that duct function may not be directly regulated by the secretagogue.

L37 ANSWER 48 OF 53 MEDLINE on STN DUPLICATE 27

AB The effect of cyclic GMP (cGMP) on human platelet activation was investigated, using its metabolically stable analogue, 8-bromo cGMP (8-bcGMP). Thrombin-induced serotonin secretion was inhibited by pretreatment with 8bcGMP in a dose-dependent manner. Production of inositol trisphosphate (IP3), a Ca2+ releaser was inhibited by 8bcGMP pretreatment of platelets. Preincubation of platelets with 8bcGMP was without effect on the basal level of cytosolic free Ca2+, measured by **fluorescent** indicator quin2, but suppressed its thrombin-induced enhancement independently of extracellular Ca2+. These results **indicate** that **cGMP** may be implicated in phospholipase C activation and Ca2+ mobilization (both influx through the plasma membrane and efflux from internal stores) in thrombin-activated human platelets.

L37 ANSWER 49 OF 53 MEDLINE on STN DUPLICATE 28

AB By employing a combination of highly sensitive radioimmunoassays and histochemical techniques, an in vivo time course of cGMP levels has been determined in the outer segment, photoreceptor cell and outer plexiform layers of frog retina. Frogs (Rana pipiens) were dark-adapted overnight and either frozen rapidly (approximately 3 sec) in liquid nitrogen or exposed to periods of light varying between 0.1 sec and 2 hr before freezing. Frozen retinal sections were cut, freeze-dried, and samples of individual layers dissected out and analysed for cGMP. In the outer plexiform layer, there was a 42% drop in cGMP concentration after 2 sec of light (250 ft candles) followed by a 34% rise after 2 min; a steep concentration gradient formed around the layer after the 2 min exposure. In both the outer-segment layer and photoreceptor-cell layer (which includes outer segments, inner segments and outer nuclear layers), cGMP levels declined from a dark value of 56 mumol kg-1 (dry) to 9 mumol kg-1 (dry) as a result of increasing exposure to several types of light source: levels appear to be primarily a function of total ft candle min. Cyclic GMP concentrations at the longest exposures (2 min with a fiber optic light source or 2 hr with **fluorescent** room light) reached identical minimum levels. In the outer segments, a 15% decrease in cGMP was observed after 0.1 sec of light exposure. Although the freezing time is too long to be able to say whether the 15% decrease in cGMP at the 0.1 sec exposure is involved in transduction, the low identical levels reached gradually after longer exposures appear to **indicate** that a light-induced biochemical adjustment in **cGMP** metabolism occurs over a relatively long time period separate from the msec time course of the transduction process.

L37 ANSWER 50 OF 53 MEDLINE on STN

AB The synthesis of **fluorescent** derivatives of cAMP and cGMP, by reaction with isatoic anhydride in aqueous solution at mild pH and temperature, yielding 2'-O-anthraniloyl derivatives of cyclic nucleotides, is here described. 2'-O-(N-Methylanthraniloyl) derivatives were also synthesized by reaction with N-methylisatoic anhydride. Upon excitation at 330-350 nm, these derivatives exhibited maximum **fluorescence** emission at 430-445 nm in aqueous solution with quantum yields of 0.11-0.26. Their **fluorescence** was sensitive to the polarity of solvent; in N,N-dimethylformamide quantum yields of 0.8-0.95. The major differences between the two fluorophores were the longer wavelength of the emission maximum of the N-methylanthraniloyl group and its greater quantum yield. The derivatives were substrates for beef heart cyclic nucleotide phosphodiesterase, 15-24% as effective as the natural substrate cAMP. When combined with thin layer chromatography techniques, two apparent Km values (3-4 microM and 36-76 microM) for the cAMP derivatives and one value (10-18 microM) for the cGMP derivatives were obtained. The results **indicate** that these 2'-hydroxyl-modified cAMP and **cGMP** can be useful **fluorescent** substrate analogs for cyclic nucleotide phosphodiesterase.

L37 ANSWER 51 OF 53 MEDLINE on STN DUPLICATE 29

AB The distribution of cyclic GMP-dependent protein kinase in rat brain has been studied by an immunological approach involving radioimmunoassay and **fluorescence** immunohistochemistry. Data obtained by radioimmunoassay **indicate** that **cyclic GMP** -dependent protein kinase is 20- to 40-fold more concentrated in cerebellum than in other brain regions. Immunohistochemical experiments demonstrate that the high concentration of immunoreactivity of the protein kinase in cerebellum is attributable to Purkinje cells. Immunoreactivity in these cells is homogeneously distributed throughout the cell (perikarya, dendrites, and axons) with the exception of the nucleus. No other neurons either in the cerebellum or in other brain regions were stained by antiserum to the protein kinase. Immunoreactivity, however, was found throughout the brain on smooth muscle cells of blood vessels.

L37 ANSWER 52 OF 53 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

DUPLICATE 30

AB Cyclic CGMP microscopy immunoreactivity in the rat's cerebellum was studied with light and EM by the indirect **fluorescence** method and the peroxidase-antiperoxidase method. Labeled cells included neuroglial cells in the cerebellar cortex, white matter and deep nuclei; some stellate and basket cells in the cortex; and some large neurons in the deep nuclei. No evidence was found for sagittal microzonation in the cGMP distribution. In the labeled cells, cGMP immunoreactive sites were localized to surface membranes, organelles and the cytoplasmic matrix. Specificity was **indicated** by the same pattern of labeling after treatment with **cGMP** immunoglobulin that had been adsorbed with cyclic(c)AMP and by the failure to label after treatment with normal rabbit sera or with cGMP immunoglobulin that had been adsorbed with 1 mM cGMP. Cerebella treated with cAMP antisera showed immunoreactivity in Purkinje cells, granule cells and Golgi cells in addition to neuroglia in cortex and deep nuclei. Sequential norepinephrine and glutamate superfusions generally intensified cGMP immunoreactivity, not only in neuroglial cells but also in the background. Under these conditions some Purkinje cells and some granule cells were also labeled. Increased cGMP immunoreactivity was also obtained by treatment with harmaline, GABA and aminooxyacetic acid, muscimol or apomorphine in order of decreasing effectiveness. Serotonin and colchicine produced no detectable increase of cGMP immunoreactivity above normal, and diazepam and sodium pentobarbital decreased it. In these experiments, diethyl ether was preferable to sodium pentobarbital for anesthesia on account of the depressive action of the latter on cGMP immunoreactivity. Drugs that increase cerebellar activity enhance cGMP levels, whereas those that decrease cerebellar activity decrease cGMP levels. It is unclear whether these fluctuations in cGMP levels are a direct consequence of neurotransmitter function or are sequelae to other related events. Some neurons and many neuroglial cells are the major sites of cGMP in the cerebellum.

L37 ANSWER 53 OF 53 MEDLINE on STN DUPLICATE 31

AB Immunohistochemical studies employing antibodies against cyclic nucleotides **indicate** that cyclic AMP and **cyclic GMP** are localized to distinct subcellular sites. These antibodies, however, cross-react weakly with noncyclic nucleotides (eg. ATP, GTP), and therefore we investigated the speficity of the immunohistochemical technique. Slides of fetal nuclei exposed to gaseous nitrous acid demonstrated reduced immunofluorescence. The slides were then incubated with cyclic and noncyclic nucleotides, and restoration of distinct cyclic AMP and cyclic GMP staining pattern was achieved only with appropriate cyclic nucleotides. Antibodies that were used have a greater affinity for acetylated derivatives of cyclic nucleotides. By using a gas phase technique, tissue slices were acetylated and immunohistochemical staining intensity was compared with the effect of acetylation on antibody affinity for various nucleotides. Acetylation greatly increased affinity of cyclic AMP antibody for cyclic AMP but not other nucleotides, and greatly intensified cyclic AMP staining. Acetylation moderately increased affinity of cyclic GMP antibody for cyclic GMP, and moderately intensified cyclic GMP staining. Conclusion: Both nitrous acid and acetylation studies support the specificity of the immunohistochemical method for cyclic nucleotides.

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